Biodiversity and Productivity relationships: experimental approaches using intertidal macroalgal assemblages

Sonia Rodríguez Benítez
Doutoramento em Biologia
Departamento Biologia
2016

Orientador
Doutor Francisco Arenas, Investigador, Ciimar (Porto)

Coorientador
Professora Doutora Isabel Sousa Pinto, Fcup-Ciimar (Porto)

Agradecimentos

Queria agradecer a todas as pessoas que de uma forma ou de outra contribuíram para realização desta tese.

Em primeiro lugar gostaria de agradecer aos meus orientadores Doutor Francisco Arenas e Professora Doutora Isabel Sousa Pinto por me aceitarem como estudante de doutoramento. Obrigado pelo apoio durante estes anos. Ao meu orientador Francisco agradeço a ajuda, a paciência e a sua entrega durante todo este período. Gracias Paco!

A investigação deste projecto não teria sido possível sem o financiamento da Fundação para a Ciência e Tecnologia (FCT) através de uma bolsa de doutoramento (SFRH / BD /66791/2009). Obrigado também ao Ciimar (Centro Interdisciplinar de Investigação Marinha e Ambiental) por disponibilizar as suas instalações durante estes anos de trabalho.

Aos meus pais e família, sem eles este trabalho não seria possível, a quem agradeço tudo o que fizeram por mim, toda a disponibilidade e todo o amor que fizeram de mim a pessoa que sou hoje. Mesmo estando numa ilha no Atlântico estiveram sempre perto.

Ao meu namorado,Airam, pelo apoio, ajuda em todos os momentos e pela paciência durante estes anos. Esta tese também é tua, Muito Obrigado! Agora um novo caminho!

A todos os que passaram pelo nosso laboratorio do Ciimar, companheiros e amigos em geral, obrigado pela amizade, solidariedade e disponibilidade nos momentos mais stressantes (Mafalda,Angela,Ana Raquel, Agnés,Andreia,David, María José, Marta A.,Helena, Duarte,João, Alba, Paloma,Ricardo,Sofía,Tánia,Luci,Carla, Laura …e alguns mais).

Aos meus “bandoleros” (Patrícia, Raquel, Diogo,Angela, Airam) por estarem sempre presentes, pelos momentos de diversão e trabalho de campo, obrigado meninos! We can!

A meus amigos de sempre que estão longe pero muito presentes (Lucía,Ruth, Eva,Carlos,Airam,Coro,Borja,Jéssica, Mireia, Saioa,Herineiva, Itahisa, Zenayda...são muitos...)

À Olga porque foi um pouco a culpada de tudo isto, estas sempre aqui!

Ao Iván pela ajuda e sobretudo pelo apoio, porque mesmo chegando tarde chegou no bom momento!

Ao Aldo obrigado pelos seus conhecimentos.

Aos meus profesores de dança (Magda Jalkuska e Joana Oliveira) por todo o carinho, apoio e por me fazerem sentir entre amigos.

♡

Obrigado a todos!

Summary

The overall loss of biodiversity has prompted considerable research on the functional consequences of biological diversity and of its loss. In this context a new understanding of the biodiversity has emerged. Biodiversity is no longer viewed as a passive consequence of abiotic and biotic interactions but as a major driver of the functioning of ecosystems. However our current understanding of how different components of biodiversity beyond species richness, for instance evenness, spatial aggregation etc…, regulates natural ecosystems function is still very fragmented and incomplete. Additionally other concerns include the relevance of the experimental systems that are often used as experiments with randomly selected species.

In the present thesis I examined different aspects of the role of diversity in the functioning of intertidal rocky-shores ecosystems incorporating some non-random patterns generally described in natural assemblages.

First, in **Chapter 1**, a general overview of the research carried out into the relationship between biodiversity and ecosystem functioning is presented. This brief review includes those singularity and achievements of marine research in this area.

The following chapter, **Chapter 2,** includes an experimental study where the interaction between species richness and evenness on synthetic macroalgal assemblages is examined. We used a modification of the design proposed by Benedetti-Cecchi in 2004 to examine the functional consequences of these two diversity-related traits while controlling from the potentially confounding effect of species identity. Results suggest that evenness may be a very relevant functional driver on low diverse assemblages. In **Chapter 3** propose an approach to study *in situ* the functional effects of induced disturbance on natural macroalgal communities from rockpools, where most of the previous research has focused on the structural effects of disturbances. We performed two different experiments where physical disturbance was applied onto natural rockpools assemblages. Results showed contrasting effects, both structural and functionally, on the two experiments. Thus, contingent variables like community traits, seems to be very relevant in the effects of disturbance. In **Chapter4** we assess the functional effects of one of the coexistence mechanisms in natural assemblages: successional diversity. Specifically, this chapter explores how successional dynamics modifies relationships between diversity and ecosystem functioning on macroalgal intertidal naturals communities. Two different successional scenarios were included in this study: primary and secondary succession. To test both scenarios we setup an experiment where relationships between primary productivity and two biodiversity

proxies (species richness and evenness) were measured. Examining the results globally, a positive diversity trend in most of the examined functional proxies was found. However the coexistence mechanisms driving community dynamics in primary and secondary succession seemed to produce differences in the effects of functional diversity between both succession scenarios and also between the two successional stages (early versus late succession). **Chapter 5** examined the effects of patchiness on the functioning of macroalgal assemblages. Specifically, this chapter intended to evaluate how assemblages aggregation of the species within the community would the productivity of seaweeds communities in an experimental approach which represented a novel approach for marine ecosystems studies. We found that effects of spatial heterogeneity was relevant to primary productivity, and as some authors have found on terrestrial systems highly patched communities were more productive that very aggregates assemblages.

Finally**,** in the **Chapter 6** provided a brief and general discussion of the main results of this thesis and its possible extrapolation to the general understanding of the consequences of biological diversity in to marine systems.

The work developed with this thesis contributed to move forward marine biodiversity research, helping to understand the relevance of different components of biodiversity and how diversity generating processes in natural systems, taking advantage of a very tractable marine model system: macroalgal assemblages from intertidal rockpools.

Resumo

A perda global de biodiversidade a nivel global promoveu a investigação científica acerca das consequências da biodiversidade funcional. Neste contexto surgiu uma nova forma de compreensão da biodiversidade. Até então, a biodiversidade tinha sido vista como uma consequência passiva de interacções bióticas e abióticas. Hoje, é vista como tendo um papel preponderante para o funcionamento dos ecossistemas. No entanto, no nosso conhecimento de como outros componentes da biodiversidade por exemplo, as espécies, a equitatividade, a agregação espacial, etc…regulam o funcionamento dos ecossistemas não é completo. Adicionalmente outros trabalhos incluem experiencias com espécies aleatórias selectivas.

No presente trabalho analisei diferentes aspectos do papel da biodiversidade no funcionamento dos ecossistemas intertidais rochosos, onde incorporei alguns padrões não-aleatórios geralmente descritos para comunidades naturais.

Primeiro, no **Capítulo 1**, efectuei uma análise geral da investigação realizada na relação entre biodiversidade e o funcionamento do ecossistema. Uma breve revisão que inclui o que foi analisado e os avanços científicos nesta área de investigação marinha. No capítulo seguinte, **Capítulo 2**, incluí um estudo experimental onde se analisa a interacção entre riqueza de especies e a equitatividade nas comunidades sintéticas de macroalgas. O desenho proposto por Benedetti-Cecchi in 2004 foi modificado para examinar as consequências funcionais destas duas características relativas à diversidade enquanto controla o potencial efeito de confusão na identidade de espécie. Os resultados sugerem que a equitatividade poderá ser relevante como indicador funcional para comunidades pouco diversas. O **Capítulo 3** propõe uma abordagem *in situ* dos efeitos funcionais de perturbações físicas induzidas em comunidades naturais de poças de maré. Trabalhos de investigação anteriores focaram-se essencialmente nos efeitos estruturais da perturbação. Realizaram-se duas experiências diferentes onde a perturbação física foi aplicada em comunidades naturais de poças de maré. Os resultados mostraram efeitos contrastantes, estruturais e funcionais. Assim, variáveis contingentes como as comunidades características parecem ser bastante relevantes no efeito da perturbação. No **Capítulo 4**, o objetivo foi avaliar os efeitos funcionais de um dos mecanismos de coexistência em comunnidades naturais: a diversidade suceccional. Especificamente, este capítulo explora como a dinâmica sucessional modifica as relações entre a diversidade e o funcionamento dos ecossistemas intertidais de comunidades de macroalgas naturais. Dois diferentes cenários sucessionais foram incluídos neste estudo: a sucessão

primária e a secundária. Para testar ambos cenários desenhou-se um trabalho experimental que nós da as relações entre produtividade primária e dois componentes da biodiversidade (riqueza de espécies e equitatividade) que foram medidos. Analisando globalmente os resultados, verifiquei uma tendência positiva de diversidade para a maioria dos componentes funcionais estudados. No entanto, os mecanismos de coexistência que conduzem a dinâmica da comunidade na sucessão primária e secundária parecem produzir diferenças na diversidade funcional assim como efeitos entre os dois tipos de cenários sucessionais e também entre a etapa sucessional (anterior versus sucessão posterior). O **Capítulo 5,** analisa os efeitos da agregação espacial no funcionamento das comunidades de macroalgas. Especificamente, este capítulo propõem avaliar como a agregação de espécies na comunidade poderá afectar a produtividade das comunidades de macroalgas. Este estudo experimental apresenta uma simulação para o estudo dos ecossistemas marinhos. Conclui-se que os efeitos da heterogeneidade espacial são relevantes para a productividade primaria e assim como alguns autores descreveram em comunidades terrestres, elevados padrões de mosaicos são mais produtivos quando comparados com comunidades agregadas.

Finalmente no **Capítulo 6** proporciona uma breve e geral discussão dos resultados principais desta tese assim como a sua possível extrapolação para o conhecimento geral das consequências da diversidade biologica nos sistemas marinhos.

O trabalho desenvolvido no âmbito desta tese contribuiu para o avanço da investigação na área de biodiversidade marinha, ajudando na compreensão da relevância das diferentes componentes da biodiversidade e dos processos geradores de diversidade em sistemas naturais, a partir de um acessível modelo marinho, como é o caso das comunidades de macroalgas de poças intertidais.

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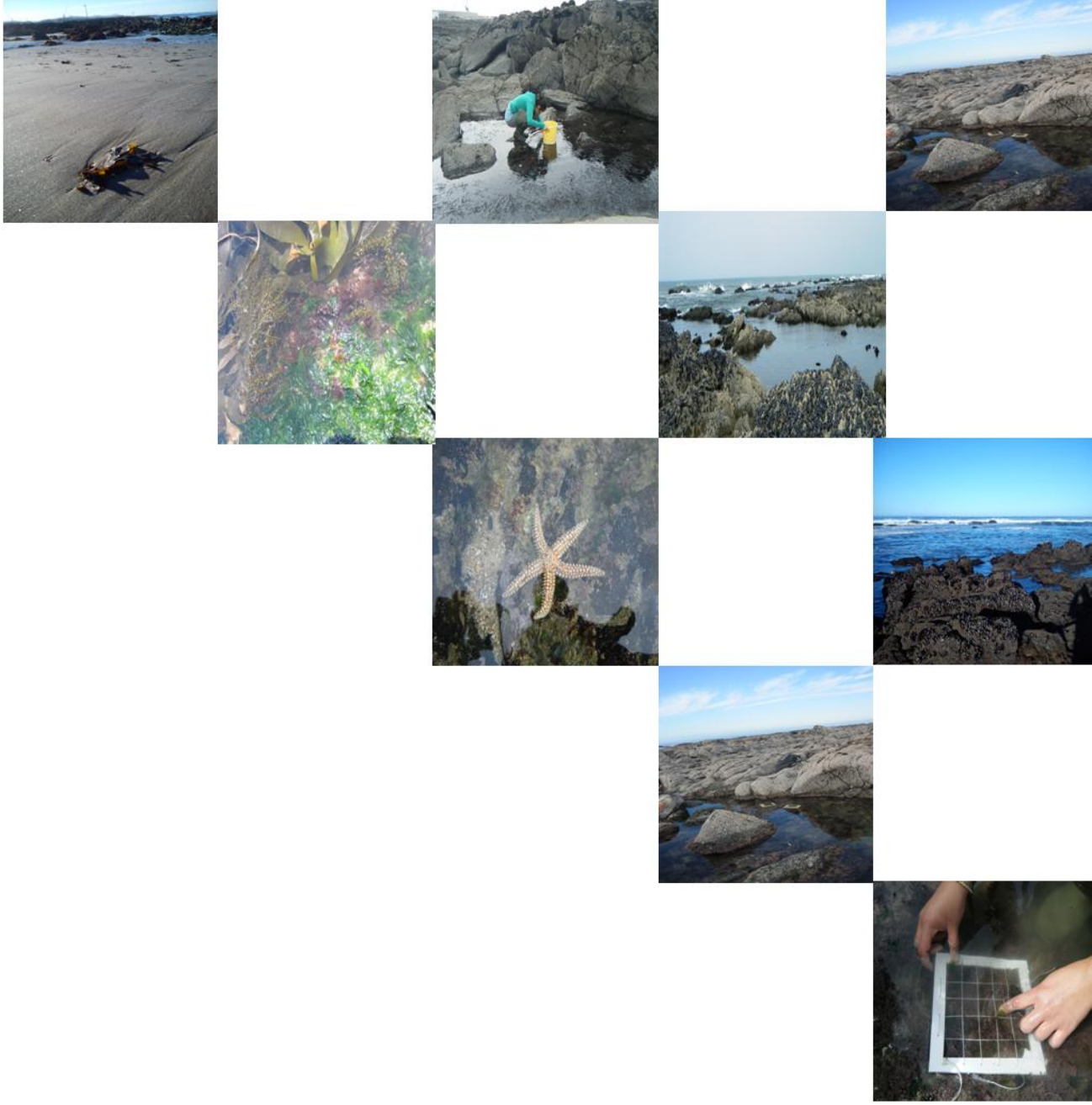
Abbreviations

IUBS/SCOPE	Unesco Programme for Biological Diversity
BEF	Biodiversity Ecosystem Functioning
DW	Dry Weight
FW	Fresh Water
ANOVA	Analysis of variance
EV	Evenness
Id	Identity
SR	Species Richness
R	Richness
spp	species
LRR	Log Response Ratios
SLA	Specific leaf area
LDMC	Leaf dry mass content
LT	Leaf thickness
VS	Frond length
FD	Functional diversity index
FDis	Functional dispersion index
CWM	Community weighted
CMWs	Indexes within communities
GPP	Gross Primary Productivity
NPP	Net primary Productivity
Resp	Respiration
Alpha (α)	Light photosynthetic efficiency
MRH	Mass Ratio Hypothesis
IDH	Intermediate disturbance hypothesis
LD	Low disturbed
HD	High disturbed
P-I	Productivity-Irradiance

LMM	Mixed models
MAMs	Minimum adequate models
AIC	Akaike Information Criterion
D	Disturbed
C	Control
Ha	half plate
Qu	Quarters of plate
Ch	Chess



Part I

Chapter1

General Introduction

"*Nature is not fragile…what is fragile are the Ecosystem services on which humans depend*"
Levin, 1999.

1.1. Biodiversity loss

Over the last decades, human impacts on ecosystems are increasing in scale and magnitude (Cardinale,2012). Large scale impacts like climate change, overexploitation of natural resources and the destruction and fragmentation of natural habitats are predicted to cause unprecedented changes in the global environment, altering biodiversity (Chapin et al., 2000). The general loss of diversity currently underway has generated concern for many reasons, ranging from aesthetic and spiritual to purely commercial. But from the most practical perspective, perhaps the most relevant concerns involve the potential consequences of species loss for the continued functioning of ecosystems and the services they provide to humanity (Duffy, 2009). For instance, ecosystem properties which are closely related with global geochemical processes, like productivity, decomposition rates and nutrient cycling, are disturbingly modified at increasing rates and scales as a consequence of the steady global reduction of diversity (Loreau et al., 2001), causing also economic impacts by affecting ecosystem products and services (Turner et al., 2007). Hence, one of the key questions now is how much loss of biological diversity can the ecosystem cope with while providing society with the goods and services needed to prosper (Cardinale, 2012). From experimental studies Hooper and collaborators (2012) estimated that species loss levels between 21 and 40 % would reduce primary productivity by 5-10%. Scenarios of species loss so intense are improbable; however local random species loss like those simulated in most of those experiments usually underestimate the effects of declining diversity, since experiments have used randomly selected assemblages from a local species pool to construct diversity gradients. It is therefore difficult, to predict the functional consequences of realistic declines in biodiversity. (Bracken et al., 2008). Thus, to estimate real-world impacts of losing diversity it is necessary to examine not only the differences in the functional performance among species, but also the differential species' susceptibility to become locally or regionally extinct.

Cardinale et al., (2012) recently summarized the current evidences gathered in the last two decades on the impacts of biodiversity loss and concluded four “consensus statements” based on the published evidences: i) There are unequivocal evidences that biodiversity loss reduces the efficiency by which ecological communities capture

biologically essential resources, produce biomass, decompose and recycle biologically essential nutrients, ii) Biodiversity also increases stability of ecosystems, iii) The impact of biodiversity on any single ecosystem process is nonlinear and saturating and iiii) diverse communities are more productive because they include species with large influence on productivity (sampling effects), and differences in functional traits among organisms increase total resource capture or facilitative interactions among species (complementarity effects).

In the marine realm more than 40 % of the ocean surface is heavily affected by human activities (Halpern et al., 2007) and the consequences of lossing diversity may be even higher than expected (Mora et al., 2011). Human impacts have pushed estuarine and coastal ecosystems far from their historical baseline of rich, diverse, and productive ecosystems (Lotze et al., 2006); depleting > 90% of formerly important species, destroying 65 % of seagrass and wetland habitats and accelarating biological invasions. All these effects are the result of synergistic effects of stressors such as habitat destruction, overfishing, invasions, warming, acidification, toxins, and eutrophication which are transforming once complex ecosystems like coral reefs and kelp forests into monotonous level bottoms, altering clear and productive coastal seas into anoxic dead zones, and transforming complex food webs topped by big animals into simplified, microbially dominated ecosystems with boom and bust cycles of toxic dinoflagellate blooms, jellyfish, and disease (Jackson, 2008).

Only intensifiying our efforts to slow climate change, rebuild affected populations and habitats, and intelligently engage the coming wave of new marine development activities will all help to change the present course of marine loss of species (McCauley et al., 2015).

1.2. Biodiversity and ecosystem functioning

The concepts of biodiversity and ecosystem function are not new to ecology even though the study of the relationship between them is relatively recent (Naeem, 2002). The concept of biodiversity emerged in the early 1980s (Harper & Hawksworth,1995) to refocus attention on the earth's biota from a more inclusive perspective. Unlike taxonomic diversity, biodiversity includes genetic and ecological diversity across all scales (spatial, temporal, and biotic scales of organization ranging from cells to ecosystems). Formally, biological diversity (i.e. biodiversity) was defined in 1992 by the

Convention of Biological Diversity as “the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems”.

Ecosystem function merge two different concepts that have been increasingly linked in the last years. Tansley (1935) coined the term ‘‘ecosystem’’ partly because he felt that ‘‘natural human prejudices’’ tend to focus attention on organisms rather than on the system as a whole Aber and Melillo (1991). Jax (2005) reviewed the concept of function which he equalled either to process or groups of processes, but it is also used sometimes as synonymous of role in connection with the idea of functional types (e.g. primary producer). Finally this author wrote that functions are also frequently implied in the context of ‘‘ecosystem services’. Thus, ecosystem function is associated with the idea that the collective metabolic activities of organisms within a habitat consume energy and move matter between organic (dead or living) and inorganic pools. Ecosystem function can be defined as the ecological processes that control changes of energy and matter over time and space, driven both by biotic activities as well as by abiotic factors, i.e. physical and chemical backgrounds (Reiss et al., 2009). Ecosystem function includes primary production, biomass accumulation, decomposition rates, nutrient use, bioturbation, just to name a few (Schwartz, 2000) but can also be represented by measures of ecosystem resilience and stability (Srivastava & Vellend, 2005).

In June 1989, in a meeting of the IUBS/SCOPE program (UNESCO Programme for Biological Diversity) held at the National Academy of Sciences in Washington and named as “Ecosystem Function of Biological Diversity”, both concepts appeared formally together probably for the first time. However the link was not new. In 1859, Darwin already hypothesized that diversity should affect production. Later the potential relationships between diversity, stability and invasibility were further explored by ecologists like MacArthur (1955), Odum (1969) or Elton (1958).

But it was in the early 1990s, when an increasing number of ecologists began to challenge the view that biodiversity was ruled by the abiotic conditions of the environment and recognized that instead properties of ecosystems are also mediated by the species present. Compared to the traditional concept of biodiversity as the result of synergistic environmental factors and species interactions, biodiversity was seen as an inherent property of ecosystems that largely governs its functioning (Naeem, 2002). The development of this central tenet was formalized at a conference held in Bayreuth, Germany in 1991 and later compiled in the book “Biodiversity and Ecosystem functioning” edited by Schulze and Mooney (1993). This book was the first milestone summarizing the knowledge on the relationship between biodiversity and ecosystem functioning, mainly based on results from comparative studies. It led to an extended range of hypotheses that collectively formed a framework for experimentally driven modern research on the issue.

To investigate the effects of biodiversity on ecosystem processes, researchers developed a new experimental approaches in which they manipulated species richness using synthesized model assemblages first in terrestrial (Tilman, 1997) and later also in aquatic environments, see (Bruno et al., 2005, Bruno et al., 2006). Those first studies focused mainly on effects of plant taxonomic diversity and plant functional-group diversity on primary production and nutrient retention in grassland ecosystems (e.g., (Tilman et al., 1997; Hooper & Vitousek, 1997; Hector, 1998), and emphasized the importance of niche complementarity and species diversity in governing ecosystem properties, especially productivity (Tilman,1999). Functional group diversity and species diversity were often manipulated simultaneously in biodiversity experiments (e.g. Tilman,1997;Symstad,2000). Researchers quickly realised the relevance of functional traits diversity, which was noticed as a more powerful predictor for ecosystem functions than species diversity (e.g. Díaz and Cabido, 2001). At this early stage of BEF (Biodiversity Ecosystem Functioning) research, the relationship between biodiversity and primary production was intensely debated. First in regard to whether community diversity depends on production (Grime ,1997, Huston, 1997) or production depends on diversity (Vitousek & Hooper, 1993; Naeem et al., 1994), and secondly because the relative importance of the mechanisms behind the observed relationships. Theoretical work and experimental studies early found an enhancement of primary production with increasing plant diversity (Loreau et al., 2001) and the mechanisms started to be defined (Tilman ,1999).

The mechanisms suggested to explain this relationship was complementary resource use (Hooper,1998) and facilitation (Cardinale et al., 2002) The ‘complementarity effect’, commonly invoked as the most relevant mechanism, occurs because resource partitioning or facilitation among species leads to increased total resource use in more diverse communities (Tilman et al.,1997, Loreau et al., 2001). Alternatively, a positive diversity effect could result from a ‘selection effect’, in which diverse communities perform better due to their greater likelihood of containing high-yielding species (Aarssen,1997, Huston,1997, Tilman,1997). One of the major debates in BEF research was focused on the relative contribution of 'selection effects' and 'complementarity effects' to the enhancement of production (Loreau,1998a, b). In the 'selection effect', dominance by species with particular traits, such as high production or biomass which disproportionately affect ecosystem processes, whereas in the `complementarity effect', resource partitioning among species or positive interactions lead to increased total resource use (Loreau & Hector, 2001, Loreau et al., 2001). The mechanisms by which ecosystem function is potentially enhanced with increasing diversity (Figure1.1)

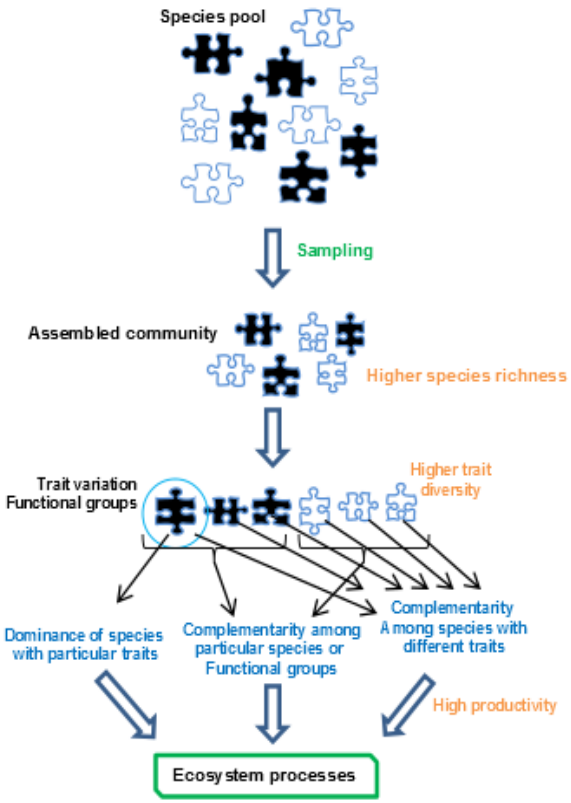


Figure1.1.Description of the contribution of sampling effects and complementarity on ecosystem processes (from Loreau et al.,2001).

In 2005, Hooper and collaborators published a large comprehensive review on the progress done to date on the biodiversity- ecosystem functioning (BEF) research (Hoper et al., 2005). Their main conclusions highlighted the relevance of species functional trait on ecosystem properties and processes. They also pointed out on the consequences of extinctions and invasion on ecosystems services and goods with some well documented examples. However diversity loss impacts are often species and ecosystem dependent, making predictions difficult.

As the several reviews published in the last few years corroborate (Cardinale, 2012, Hooper et al., 2012; Tilman et al., 2014), research on biodiversity ecosystem functioning has reach maturity and results are consistent and always point the same perturbing direction. Hence the recent reviews published by Hooper and colleagues (2012) and Tilman and colleagues (2012) using different data sets to compare the impacts of plant diversity versus other factors on primary productivity, found that decreases in plant biodiversity of the magnitude imposed by human actions had an impact as large as or larger than other equally relevant disturbance like nitrogen deposition, elevated CO_2, fire, herbivory, and drought. Hooper et al., (2012) concluded that their “analyses clearly show that the ecosystem consequences of local species loss are as quantitatively significant as the direct effects of several global change stressors that have mobilized major international concern and remediation efforts”

The second review by Tilman et al., (2012), stated that “changes in diversity of the magnitude being imposed by human actions (e.g. overexploitation, eutrophication, pollution, and species introductions) can have at least as great of an effect on primary productivity as anthropogenic changes in atmospheric CO_2, the availability of a limiting soil resource, herbivory, fire, and variation in water availability”. It concluded by saying that “contemporary biodiversity declines are among the dominant drivers of changes in ecosystem functioning”.

To fully understand the consequences of biodiversity on ecosystem functioning there are however some aspects that should be further investigated. Many of the experimental studies done were based on microcosms or mesocosms units, where diversity was manipulated by randomly choosing some number of species from a pool of species, placing these together in experimental units then examining the relationship between species diversity and the ecosystem processes of interest. These studies have helped greatly to articulate the hypothesis of the functional consequences of biodiversity, providing important insights into the nature of the BEF relationship and its

underlying mechanisms. However, it is uncertain to what extent these results can be extrapolated to natural systems. In fact, the ability of experimental studies to assess the importance of biodiversity for ecosystem functions has been debated, as ecosystem functions in natural systems are rarely affected by biodiversity alone (Huston & Mcbride, 2002; Srivastava & Vellend, 2005; Naeem et al., 2012). For example, disturbances that structure biodiversity patterns are likely to impact ecosystem functions directly, and alter the BEF relationship. Understanding how the loss of a species or functional group affects the functioning of the wider ecosystem is vital if we are to lessen or mitigate the impacts of anthropogenic disturbance. Concerns about the generality for real communities of our current understanding of biodiversity-ecosystem functioning relationships also derives from the fact that some diversity-promoting mechanisms like immigration processes, competition-colonization trade-off in successional transitions, and disturbance regimes may not produce the described positive effect of diversity (Cardinale et al., 2000; Mouquet et al., 2002). Thus mechanisms through which species coexist may explain why the trajectory and magnitude of the biodiversity-ecosystem functioning relationship can be complex and variable. Furthermore, spatial distribution of the species in experimental plots is typically random. However, in natural communities, spatial distribution of plants and animals is typically non-random. These spatial patterns may be relevant to ecosystem functioning (Maestre et al., 2005; Arenas et al., 2009)

The current literature on marine species richness and ecosystem functioning has a clear message: losing species will cause general reductions in ecosystem functions on average (see also Stachowicz et al., 2007; Worm et al., 2006). However, compared to the biodiversity functioning field as a whole, the sample size for particular marine systems and function categories is low. Although most of the evidence points to an enhancement of function with diversity in marine ecosystem like the terrestrial environment, the marine environment remains largely enigmatic due to its large size and taxonomic complexity (Worm et al., 2006) and some unexpected results have already been discovered (Loreau, 2008; Gamfeldt et al., 2014). In a recent review of the biodiversity-ecosystem research done on marine systems, (Gamfeldt et al., 2014) examined 174 experiments and found consistent results with terrestrial literature. Losing of species will, on average, tend to alter the functioning of marine ecosystems. However marine ecosystems differ fundamentally from their terrestrial counterparts and unexpected findings on biodiversity-ecosystem functioning relationship have been already published (Danovaro et al., 2008). Marine systems are relatively open, with

fewer limits to organism dispersal and energy flow, while terrestrial systems are relatively closed and material flow is largely local (Giller et al., 2004). Furthermore, many of the studies have focused on higher trophic levels such as grazers (Duffy et al., 2001) or in faunal invertebrate assemblages (Emmerson et al. 2001; Solan et al., 2004) with only less profuse studies testing primary production of aquatic primary producers.

1.3. The role of macroalgae assemblages in biodiversity-ecosystem functioning relationship research

As indicated in the previous section, research on biodiversity-ecosystems was initially led by terrestrial researchers with experiments on plant diversity–productivity relationships and the mechanisms behind them (Tilman,1999). Marine ecologists lagged slightly behind and focused more on the role of consumer diversity (Gamfeldt, 2009; Gamfeldt et al., 2014). Nevertheless, seaweed assemblages (at least in intertidal zones) are easy to manipulate, thus they are a convenient model system for experiments examining causal links between diversity and functioning because of the very small spatial and temporal scales of variability.

Research performed involving seaweeds within the context of BEF studies used similar functional proxies that terrestrial experiments, i.e. primary production, nutrient uptake, resistance to invasion and stability. Irrespective of the response measured, most of these experiments found positive effects of seaweed diversity. Thus, positive diversity effect were found in photosynthetic rates (Middelboe & Binzer, 2004, Bruno et al., 2005, Griffin et al., 2009), biomass accumulation (Bruno et al., 2005), nutrient uptake (Bracken & Nielsen 2004); invasion resistance (Arenas et al., 2006) and stability (Stachowicz et al., 2008, Boyer et al., 2009). Also, experiments suggested that diversity effects seem to be strongest when multiple ecosystem functions were jointly included in the experimental design; e.g. productivity and stability (Boyer et al., 2009) or productivity and nutrient uptake (Bracken & Williams, 2013).

Mechanisms invoked as driving those patterns were the same as in terrestrial experiments. Hence, niche differentiation leading to complementary resource use, facilitative interactions among species and species identity effects were identified as underlying mechanism governing the diversity and productivity of seaweed communities. In short term experiments the species identity appeared to be the dominant effect (Bruno et al., 2006; Griffin et al., 2009), while in longer experiments

mechanisms like facilitation and differential use of resources increase in importance (Chapman, 1990; Benedetti-Cecchi & Cinelli; 1995; vanTamelen, 1996; Menge et al., 2005; Bracken et al., 2008; Stachowicz et al., 2008; Arenas et al., 2009).

1.4. Experimental details of the studies carried out in this Thesis report

Experiments were carried out north the Northern Portugal seashores. This area is a biogeographic transition zone where many macroalgal species have their distribution limits (Araújo et al.,2009). In this region the rocky shore are typically composed of granite and slate platforms with abundant rockpools (Figure 1.2). This coastal area has a semi-diurnal tidal regime, with the largest tidal range of 3.5–4 m during spring tides. Seasonality is very marked in the seawater temperature with an annual average around 15 ºC (Cacabelos et al., 2013).

Rocky pools are very particular habitats with environmental conditions largely regulated by the tidal cycle (Metaxas et al.,1994). Each rock pool exhibits specific physic-chemical parameters such as temperature, salinity, oxygen, carbon dioxide and pH (Huggett & Griffiths,1986), as those vary particularly in relation to height on the shore, but also with pool size, shape and assemblage composition (Metaxas & Scheibling,1993). However, tide pools not only vary in environmental conditions but also on biological traits like the availability of food (e.g. Underwood,1984), level of predation (e.g. Connell,1970) and availability of recruits (Coleman & Brawley,2005).

Tide pools provide an ideal model system for evaluating the effects of realistic biodiversity change on functioning, as several potential factors influencing tide pool seaweed diversity (e.g., herbivory, disturbance, nutrient availability) have been well-studied. Intertidal pools are, temporarily closed systems, which enables easy to measure productivity and other relevant assemblages functional traits under ecologically relevant conditions (Bracken et al., 2008). Interestingly, on those intertidal pools, patchy macroalgal assemblages are maintained by different processes such as physical disturbance, successional transitions, predation, spatial heterogeneity, recruitment limitations, differential life histories, etc. (Chapman,1990; Benedetti-Cecchi & Cinelli,1995; vanTamelen,1996; Menge et al., 2005).

These diversity-promoting processes may not promote complementarity or facilitation among species, decoupling the relationship between diversity and ecosystem function properties. Understanding the causes and consequences of variation in marine algal diversity and their functional consequences is essential to our knowledge of energy flow, nutrient fluxes, and productivity in nearshore marine systems (Bracken et al.,2008)

Figure1.2. Intertidal rocky shore in North of Portugal.

1.5. General Aim and Objectives

In this thesis, the overall aim was to examine the role of diversity in the functioning of intertidal rocky-shores ecosystems by incorporating into experiments processes operating in the natural communities. Therefore, this study aimed to move forward marine biodiversity research, adding new and more realistic approaches to experiments which will help to understand the relevance of biodiversity for ecosystems performance in our model system: macroalgal assemblages from intertidal rockpools.

Chapter 2: Examines the effects and interactions of species richness and evenness on the primary productivity of synthetic assemblages. We used a modification of the design proposed by Benedetti-Cecchi, 2004 which included explicitly diversity and evenness while controlling the effect of identity.

Chapter 3: Tests the effects of disturbance on the primary production of macroalgal assemblages. Examines how physical disturbance may promote diversity but also prevent the existence of a relationship between diversity and ecosystem functioning.

Chapter 4: Explores how successional dynamics modifies the relationships between diversity and ecosystem functioning. Two different successional scenarios are included in the experiment approach: primary and secondary succession.

Chapter 5: Examines how assemblages aggregation, i.e. the spatial arrangement of the species within the community, may affect the productivity of seaweeds communities.

This thesis includes a general introduction and a final chapter with a general discussion and conclusions.

1.6. References

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Part II

Chapter 2

Biodiversity and productivity on macroalgal assemblages: disentangling richness, evenness and identity effects

Biodiversity effects on macroalgal productivity: identifying the role of richness, evenness and species traits

Sonia Rodriguez[1,2], Airam Prieto Martín[1], Isabel Sousa-Pinto[2,3] & Francisco Arenas[1]

[1]Aquatic Ecology & Evolution Group, Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

[2]Coastal Biodiversity Group, Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

[3]Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4150-181 Porto, Portugal

Rodríguez S, Martín A,Sousa–Pinto I,Arenas,F Biodiversity effects on macroalgal productivity: identifying the role of richness, evenness and species traits. Submitted to Marine Ecological Progress Series. *Submission id: 19916*

Abstract

In the last two decades, concerns on the effects of global loss of diversity has prompted considerable research on the functional consequences of biodiversity. In this context a new recognition of biodiversity as a major regulator on the functioning of ecosystems has emerged in ecological research. Biodiversity is a now a comprehensive concept, including species number and traits, relative abundances, etc. Understanding how these different components of biodiversity influence communities functioning is required to improve our abilities to foreseen the future consequences of current diversity losing rates. The aim of this study was to examine the relationship between diversity and ecosystem functioning in communities of rocky intertidal macroalgae, discriminating the effect of number of species, specie traits (identity) and also their relative abundance. We used a short term manipulative experiment able to disentangle the effect of the different diversity components. Results suggested that the intense identity effect, derived from species traits, drove the response of the experimental assemblages. Also, weak but consistently positive effects were also detected for evenness. The interaction between species richness and evenness suggested that the negative effect of high dominance by single species, i.e. low evenness, are exacerbated in low diverse communities. Even before loss of species occurs, changes species abundances are often the first effect of human activities in natural system. Thus, understanding the interactions between richness and evenness is critical to identify and foreseen present and future consequences of anthropogenic disturbances.

Keywords: *Biodiversity, Productivity, Species Richness, Evenness, Macroalgal assemblages*

2.1. Introduction

Organisms on Earth move hundreds of thousands of tons of elements and compounds every day through their biochemical activity (Naeem, 2002). Ecologists have investigated these physical, geochemical biological processes occurring in ecosystems for more than 50 years. Good examples are the conceptual models developed by Odum in the 1950s and 1960s characterizing the energy and matter flows through ecosystems (Odum,1969). However in the last two decades, a renewed interest on the functional attributes of ecosystems emerged as result of the recognition that under the current rates of biodiversity loss the regular functioning of Earth's natural ecosystems and the services they sustain are under threat (Hooper et al., 2005; Stachowicz et al., 2007, Reiss et al., 2009; Cardinale et al., 2012). These research efforts demonstrated that biodiversity can directly control ecosystems functions such as nutrient cycling and biomass production. Ecosystem functions are broadly defined as an amalgamation of ecosystem processes that control the fluxes of energy, nutrients and organic matter through the environment (Cardinale et al., 2012).

Despite some initial controversy regarding the mechanism underlying the relationship between biodiversity and ecosystem function (Huston, 2000; Mittelbach et al., 2001), most of the research in the last two decades hinted to similar conclusions (Loreau et al., 2001; Cardinale et al., 2006; Reiss et al., 2011; Tilman et al., 2006). Hence, a minimum number of species is necessary to maintain of functioning of ecosystems and a larger number of species is required to maintain both the assembly and functioning of ecosystems (Loreau et al., 2001). Thus, biodiversity not only determines ecosystem processes such as biogeochemical cycles, but is also relevant for ecosystem properties such as stability (Tilman et al., 2006), resistance to invasion of exotic species (Stachowicz & Byrnes, 2006) or predictability (Mcgrady-Steed et al., 1997). Three main mechanisms have been proposed as driving the positive effect of diversity (Tilman et al., 1997; Loreau et al., 2001): i) the complementary effect resulting from the complementary use of resources by different species (i.e. niche differentiation; ii) the sampling effect is a simple probabilistic effect, increasing number of species increases the probability of including highly productive species in the assemblages (Aarssen et al., 2003) Spaekova & Leps, 2001) and iii) the facilitation effect, when one organism makes the local environment more favourable for other organism, either directly, e.g. reducing thermal, water or nutrient stress via shading or through nutritional symbioses, or indirectly, e.g. removing competitors or deterring predators, (Bruno et al., 2003). The

relative importance of these mechanisms in natural systems is very much unknown (Cardinale et al., 2012; Flombaum et al., 2014).

Most of the conclusions drawn regarding the functional consequences of biodiversity are derived from theoretical models and experimental systems on which the number, identity and functional traits of species have been highly controlled at local scale (Solan et al., 2006). There has been some concern about the factual relevance of this relationship on natural systems where the distribution of abundances is not uniform across species (Schlaepfer & Schmid, 1999). Species richness alone cannot fully represent species diversity in relation to ecosystem functioning because it ignores the influence of relative species abundance on intra- and inter-specific interactions (Hillebrand et al., 2008; Zhang et al., 2012). In fact, biodiversity should be considered as a multifaceted concept which include not only the number of species (i.e., richness), but also heterogeneity (i.e. the dissimilarity among species) and evenness (i.e. the relative abundance of species in the assemblage) (Cardinale et al., 2012).

How changes on species evenness modifies ecosystem deserve some attention, because evenness usually changes more rapidly in response to anthropogenic stressors than does species richness and because evenness changes have important consequences for ecosystems long before diversity reduction happen due to species extinction (Chapin et al., 2000). Thus, species that are at risk or on the way to extirpation are probably going through a low-abundance stage before disappearing completely, and evenness may decline long before species richness does (Mulder et al., 2004). Additionally, evenness and species richness are not always correlated and both, positive and negative relationships have been described in terrestrial systems (Stirling & Wilsey, 2001). Also, richness and evenness may respond to different environmental factors or differently to a given factor reflecting different components of biodiversity (Soininen et al., 2012). The importance of understanding the functional effects of the biodiversity components including species richness, evenness, functional richness, is particularly relevant in the context of global stressors like climate change (Grimm et al., 2013) or invasions. For example Walker et al., (2006) revealed that experimental warming strongly altered species composition in tundra ecosystems across the entire circumpolar arctic region. Warmed communities had lower species richness and lower evenness than ambient controls, and thus they were strongly dominated by few species functionally very different to the natural assemblages. The arrival of introduced species is also considered to have a global impact with large

impact on native diversity assemblages. Thus, non-native species with singular functional traits are often highly dominant in their host assemblages displacing native species and modifying assemblages species and functional diversity and evenness (Ruesink et al., 2006; Wallentinus & Nyberg, 2007).

Research on the ecosystem functioning of the marine realm, lags behind terrestrial research. In marine macroalgal communities, biodiversity-ecosystem functioning investigation have mainly focused on the effects of species richness on primary production and on the stability of natural communities (Bruno et al., 2003; Stachowicz et al., 2008; Boyer et al., 2009; Kraufvelin et al., 2010; Bracken & Williams, 2013). Macroalgal communities present high rates of benthic primary production per unit of surface area stimulating very rich animal communities and high fish production in the coastal zone (Middelboe et al., 2006). Most of the studies performed to date have found significant effects of species richness on primary productivity and stability (see a recent review (Gamfeldt et al., 2015). However, the often weak relationship between species richness and evenness in aquatic systems suggests that richness and evenness often reflect independent components of biodiversity, highlighting that richness alone may be an incomplete surrogate for biodiversity (Soininen et al., 2012). One observational study linked species richness and evenness on the primary productivity of natural macroalgal assemblages (Arenas et al., 2009). In this observational study, the relationships between primary productivity and several biodiversity related traits (namely identity, species richness, evenness and spatial arrangement) were examined simultaneously in natural macroalgal assemblages. However, observational approaches have several limitations such as the impossibility to establish causality and to identify underlying mechanisms, meanwhile manipulative experimental, allow to establish causal connections between biodiversity and productivity and other measures of ecosystem functioning (Benedetti-Cecchi, 2004), and may help to understand how these structural diversity components interact to shape the ecosystem function of macroalgal communities (Arenas et al., 2009; Maestre et al., 2012).

In this study we aim to evaluate potential interactions between species richness and evenness on the primary productivity of synthetic assemblages. We created artificial macroalgal assemblages and used a modification of the design proposed by Benedetti-Cecchi (2004) including explicitly diversity and evenness as factors while controlling the effect of identity. Our initial hypotheses based in previous research is that both

evenness and species richness have positive effects on assemblages performance, with potential interactions between both factors, basically resulting from strong identity effects. To further explore the potential relationship between the different components of diversity and productivity we also examined the relationships between several functional diversity descriptors and the productivity of the assemblages.

2.2. Material and Methods

2.2.1. Synthetic assemblages and experimental design

We conducted the experiment using artificial assemblages done with eight species of macroalgae collected at the coastal area of North Portugal. These seaweeds were four brown seaweeds: *Halopteris scoparia* (Linnaeus) Sauvageau, *Bifurcaria bifurcata* R. Ross, *Fucus vesiculosus* Linnaeus, *Sargassum muticum* (Yendo) Fensholt, and four red seaweeds: *Chondracanthus acicularis* (Roth) Fredericq, *Mastocarpus stellatus* (Stackhouse) Guiry, *Chondracanthus teedei* (Mertens ex Roth) Kützing, *Osmundea pinnatifida* (Hudson) Stackhouse. All these species are abundant perennial seaweeds at the collection shores (Araújo et al., 2009), represent different morphologies and are suitable for the manipulation procedures used to create the synthetic assemblages.

Algal fronds were collected in April and May 2013 from rocky shores at Praia Norte, (41°41′27″N, 8°50′57″W) and Praia de Moledo (41°50′22″ N, 8°52′30″ W). Both sites are exposed rocky intertidal shores with large granite and slate platforms and with abundant rockpools. They have a semi-diurnal tidal regime, with the largest tidal range near to 4 m during spring tides. At the collection dates, healthy fronds from the eight different target species were cut or scraped from the rock and transferred to the laboratory in plastic bags and cool boxes. Once in the laboratory, seaweeds were sorted by species and rinsed in a bath of freshwater (about 30 seconds) to remove herbivores (Arenas et al., 2009). Fronds were them placed in separated tanks and continuously aerated seawater tanks (80 litres) (Figure2.1).

Figure2.1. Pictures of the assemblage building process. From left to right .1) Rinsing in freshwater to remove grazers. 2) Seaweed in seawater tanks. 3) Securing groups of fronds and 4) Assembling the groups on the plates assemblages.

Using the collected seaweeds, we built 96 synthetic communities with an area of 196 cm^2 each. To assemble the communities, small groups of fronds were secured with small cable ties to a 5 mm plastic mesh keeping fronds upright (Figure2.1). Groups of fronds were regularly distributed throughout the mesh with a distance among groups of around 2 cm. Overall biomass in the assemblages was 100 g of seaweeds (fresh weight, FW), within the range of biomass found in rock-pools with erected seaweeds in the collection sites (authors pers. obs.). Seaweeds and the mesh were them secured to a PVC plates to increase strength and ensure negative buoyancy (Figure2.2). Once constructed, the assemblages were submerged in four 600 l and 40 cm depth filtered seawater (salinity ~35‰) outdoor tanks set with continuous aeration to create turbulence. Tank temperature was controlled at 16ºC using Aqua Medic® Titan 2000 cooler units. To avoid nutrient limitation, seawater was enriched every two days by adding inorganic nitrogen ($NaNO_3$) and phosphorous (NaH_3PO_4) to a final concentration of approx. 50µM N and 5µM P, respectively. Salinity was regularly monitored and tanks were regularly refilled with freshwater to compensate for water evaporation.

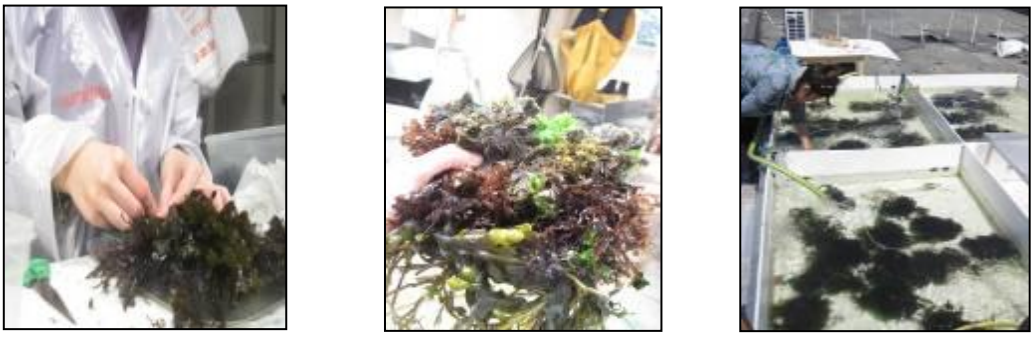

Figure 2. 2. Pictures with some assemblages and the outdoor tanks were the assemblages accommodated for two weeks.

Our experiment aimed to examine the relationships between three different components of diversity (species richness, species identity and evenness) with several proxies of primary productivity as indicators of community performance. We used a three factor mixed experimental design, adapting the design proposed by Benedetti-Cecchi (2004) (Figure 2.3). We considered three components of diversity cited above as factors of the experimental design: i) Species richness (R) as a fixed factor with two

levels (2 species and 4 species), ii) Evenness (Ev) as a fixed factor with two levels (low and high) and iii) Identity (Id), this is a random factor nested in diversity and with 4 levels, i.e. we included four different combinations of 2 species and 4 species (Figure 1, ID1, ID2, ID3, ID4). Species were randomly selected from our initial species pool to create the 2 species assemblages and 2 random additional species were added to those initial ones to produce the 4 species assemblages. Identities were: i) ID1 included the species *H. scoparia* and B. *bifurcata* in the 2 species richness treatment and 2 more species, randomly selected from the pool, in the 4 species richness treatment (*F. vesiculosus* and *S. muticum*)*;* ii) ID2 included the species *C. teedei* and *O. pinnatifida* in the 2 species richness treatment and 2 more species in the 4 species richness treatment (*C. acicularis* and *S. muticum*), iii) ID3 included the species *C. acicularis* and *M. stellatus* in the 2 species richness treatment and *B. bifurcata* and *C. teedei* in the 4 species assemblages and iv) ID4 included the species *F. vesiculosus* and *S. muticum* in the 2 species richness treatment and *O. pinnatifida* and *H. scoparia* completed the 4 species required in the high diversity treatment. Our design incorporated at the low species richness assemblages all the species from the four species richness assemblages, preventing any confounding between richness and identity effects (Bulling et al., 2006; Stachowicz et al., 2007). We created 4 replicates per identity assemblage and built a total of 64 multispecies assemblages.

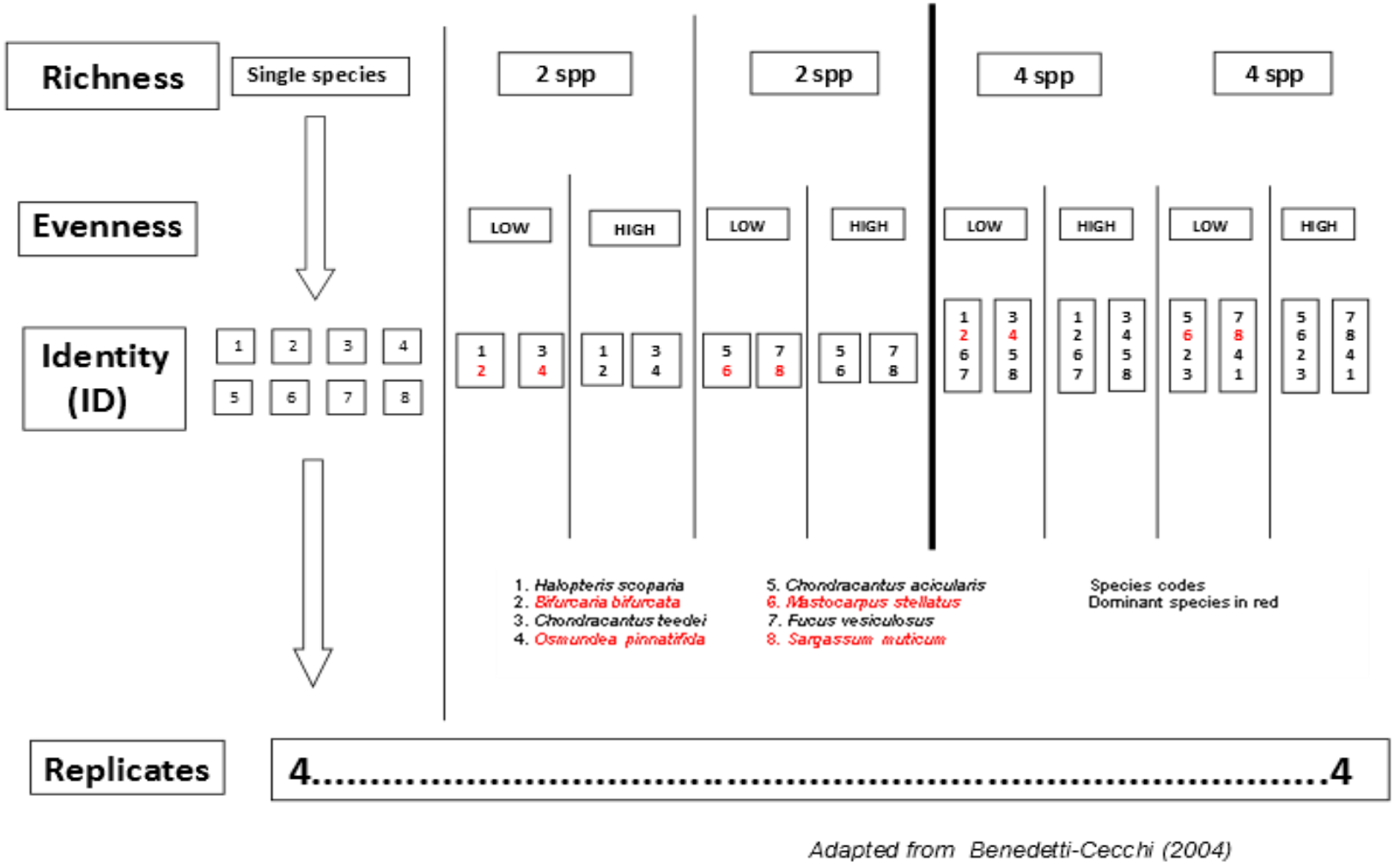

Figure2.3. Diagram of the experimental design used in our experiment to disentangle the effects of species richness, identity and evenness (distribution of biomass) using assemblages built with 8 macroalgal species. Species that occur at a high richness level also occur in treatments with low richness treatments. Dominant species in the low evenness treatment are in bold.

Evenness treatment was implemented by considering two different ways of species biomass distribution on the assemblages (i.e., high and low evenness treatments). Thus, high evenness had equal amount of biomass per specie in the assemblage while low evenness had a "dominant" species with higher biomass than the other species in the assemblage (see Figure 2.4 for a better understanding). Dominant species, randomly selected from the species pool, were: *B. bifurcata, O. pinnatifida, M. stellatus* and *S. muticum*.

A) 2 Species low evenness | B) 2 Species high evenness | C) 4 Species low evenness | D) 4 Species high evenness

Figure2.4. Evenness treatment. Different color means different algal species. Each quadrat represents a 5% of the community.

In 2-species assemblages and low evenness the dominant species included 75 g FW biomass and the non-dominant 25 g FW (Fig 2.4A). In 2-species assemblages and high evenness, each species was represented by 50 g of FW biomass (Fig 2.4B). A similar biomass splitting was in the case of 4-species assemblages, low evenness treatment; the dominant species contributed with 62.5 g FW and the other three species with 12.5 g FW each (Fig 2.4C). While in the case of high evenness treatment each species included 25 g FW (Fig 2.4D). Wet weights were determined after removing excess water from the algae using a salad spinner and a dry cloth (Bruno et al., 2005).

Additionally, we built single species assemblages for each of the eight seaweed species used in the experiment. These assemblages, 4 replicates per species, allowed examine the existence of overyielding, i.e. increased productivity in species mixtures relative to monocultures (Hooper & Dukes, 2003). A total of 96 assemblages were created for the experiment (Figure 2.3).

2.2.2. Incubation procedures and Ecosystem functioning surrogates

To allow some adjustment of the seaweeds within the assemblages, the incubations were carried out two weeks after their construction. Incubations were performed sequentially in the same order than the plates were assembled. Productivity-irradiance (P-I) curves were estimated by measuring oxygen fluxes within incubation chambers at 7 irradiance intensities (from 0 µmol $m^{-2}s^{-1}$ (dark period), 24, 164, 262, 345, 417 and 1578 µmol m^{-2} s^{-1}). Maximum irradiance levels in the chamber were lower than those recorded in the field at sea surface level where, during sunny days in winter where irradiance can reach around 2000 µmol m^{-2} s^{-1} (authors' pers. obs. using a scalar quantum sensor, Arenas et al., 2009). The light source in the chamber was composed of sixty four 30 W fluorescent tubes (Osram L® 965 Biolux). Irradiance inside the chamber was measured using a PAR sensor (Walz® ULM500 Universal Light Meter) (Figure 2.5).

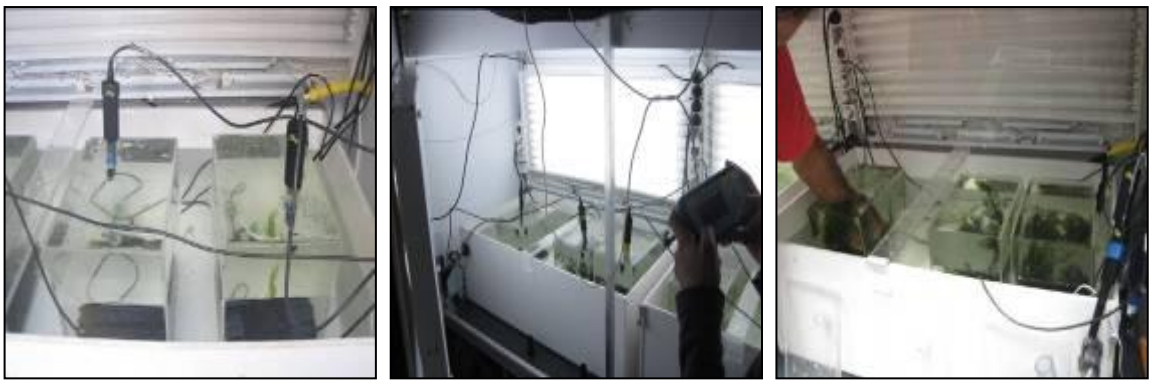

Figure2.5. Incubation procedure with the large CT chamber, temperature controlled seawater bath and the small incubation chambers where oxygen fluxes were measured.

For each P-I incubation, the successive irradiance periods lasted between 20 min, the time necessary for the fluorescent tubes to warm up and the assemblages to reach linear rates of oxygen flux (Migné et al., 2002). All the timing of the light system was controlled using Aqua Medic ® (AT Control System controllers, GmbH, Bissendorf, Germany). The entire set of incubations took around 2:30 h per assemblage. The incubation chambers consisted of 12.5 l sealed Plexiglas chambers partially submersed in a larger, temperature controlled cooling tank. Mean temperature inside the incubation chamber was 16.5± 0.06°C. We used filtered sea water and the water movement inside the incubation chambers was maintained through a submersible pump (300 l h^{-1}) equipped with diffusers to reduce turbulence. The changes through

time in oxygen concentration were measured using a luminescent dissolved oxygen probe connected to a data-logger (Hach ® HQ40) that registered a new measurement every 30 s. To reduce possible effects of circadian rhythms on algal productivity, incubations were always carried out during daylight hours (between 08:00 and 18:00h). Productivity related measures were estimated through oxygen fluxes by regressing oxygen concentration (µmol O_2 l^{-1}) through time (s^{-1}) during dark and light periods of increasing intensities. Estimations were corrected by seawater volume inside the chamber.

The variables respiration (Resp), maximum gross and net primary productivity (GPP, NPP) and photosynthetic efficiency at low light irradiance (alpha, α) were used as surrogates of assemblages functioning. Respiration of assemblages (mg O_2 h^{-1}); corresponded to the oxygen consumption rate during the dark period and net primary productivity (mg O_2 h^{-1}) was estimated from oxygen fluxes at different irradiance intensities in order to calculate alpha. Both variables were calculated by plotting oxygen concentration over incubation time and fitting a linear regression line to calculate rates of oxygen change. Alpha (α) (mg O_2 µ$Em^{-2}s^{-1}$), was estimated as the slope of P-I relationship at light-limited irradiances through linear regressions. Gross primary productivity (GPP) was calculated as the sum of NPP and the absolute value of Respiration (|Resp|) (see Figure 2.6)

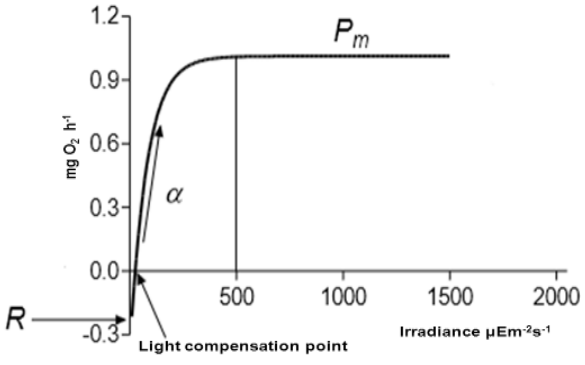

Figure2.6. Example P-I curves and the productivity variables examined. Where Pm is maximum production (NPP), α is the slope at non-saturating irradiance, R is the respiration rate at zero irradiance and the Light compensation point, where production and respiration are equal. (from Tait LW.,2010)

2.2.3. Overyielding, assemblages functional traits and diversity calculations

The relative performance of mixtures versus monospecific assemblages for each response variable were examined using log response ratios, LRR, as ln (O/E), where O is the observed response value, and E is the expected value (Orwin et al., 2014) (Matias et al., 2015). Expected values were calculated based on monoculture responses and the relative proportion of each species within the mixed communities.

Overyielding index(i+j):
Log (O (i+j)/E (i+j))

O(i+j) – *Observed primary productivity (PP) for the assemblage i+j for the corresponding spatial aggregation*
E(i+j) – *Estimated primary productivity (PP) for the assemblage i+j for the corresponding spatial aggregation*.

This ratio is an indicator of overyielding and reveals whether the functioning of a given mixture differs from the expected functioning based on the performance of the constituent species in monospecific assemblages. Thus, if LRR > 0, there is a positive diversity effect, whereas if LRR< 0 there is a negative diversity effect.
To characterize functionally each species and estimate different functional indexes for each assemblage we used a similar procedure to the one used also in seaweeds by (Griffin et al., 2009) and measured for all the species, four functional traits related to photosynthesis and resource use: i) specific leaf area (SLA), expressed in mm^2 mg^{-1}), ii) leaf dry mass content (LDMC) in mg g^{-1}, iii) leaf thickness (LT) in mm and iv) a proxy for vertical space use, i.e. frond length (VS). Measures were done in 10 fronds identical to those used to build the assemblages and collected from the same sites.

Functional diversity indexes aim to better summarize the extent of functional differences in a species pool (Ricotta, 2005) and thus should have a closer relationship to the productivity of the assemblages than other diversity descriptors like species richness. Several functional indexed have been proposed by researchers in the last years, (see Petchey & Gaston 2002; Laliberté & Legendre, 2010; Ricotta & Moretti, 2011). Thus, we calculated the functional diversity index (FD) from (Petchey & Gaston, 2002), defined by the authors as the total branch length of a functional dendrogram.

We also estimated for each assemblage the functional dispersion index (FDis), which measures the dispersion or variation of the species traits within an assemblage. This index accounts for species abundances and is unaffected by species richness. (Laliberté & Legendre, 2010). Finally we calculated the CWM, community-weighted mean trait, for each assemblage and functional trait. CMWs assess the functional structure of the assemblages using mean trait indexes within communities. CWM indexes are not properly a functional diversity index but a measure of the dominant trait value within an assemblage and are calculated as the averaged trait value in the community, weighted by the species abundance (Sydenham et al., 2016).

2.4. Statistical analyses

Analysis of variance (ANOVA) was used to test hypotheses involving productivity and all related surrogates. Changes in gross primary productivity (GPP), net primary productivity (NPP), respiration (R) and alpha were analysed using a 3-factor nested analysis of variance. Diversity (2 levels) and evenness (2 levels) were considered fixed and orthogonal factors and identity (4 levels) was considered a random factor nested in diversity. Analyses were performed on data collected after 15 days of species accommodation within the plates. Before analysis, the homogeneity of variances was evaluated with Cochran's test. Variances were not heterogeneous and data transformation was not necessary. A posteriori multiple comparisons were done using Student–Newman–Keul's (SNK) tests (α=0.05). GMAV version 5 for Windows was used for these analyses (Underwood,1997)

To further assess how the assemblage's traits may define their performance, we follow in part the methodological framework to that proposed by Díaz et al., (2007). Thus we first examined using pairwise regressions the relationships between the functional traits and diversity descriptors mentioned above and the different productivity proxies measured in the experimental assemblages. We also examined the potential idiosyncratic effects of seaweeds species by looking into the pairwise associations between species abundances and the proxies of productivity. Pairwise analyses were carried out using R (R Core Team 2015).

2.4. Results

2.4.1. Functional performance of single species assemblages

Assemblages with one single species served to characterize functional performance of each species and were used for the estimations for the log response ratios (LRR) of multiple species assemblages. All the four functional parameters estimated throughout incubations (i.e., GPP, NPP, respiration and alpha) showed significant differences among the species (ANOVA, $p<0.05$ in all the response variable models), i.e. identity effects were obvious among the selected species. *Sargassum muticum* had the highest values of GPP, NPP, Respiration and light photosynthetic efficiency (α, alpha) compared to the most of the other species. *Fucus vesiculosus*, *Chondracanthus acicularis* and *Halopteris scoparia* exhibited intermediate performances. *Bifurcaria bifurcata* was the seaweed with the lowest overall performance (Figure 2.7).

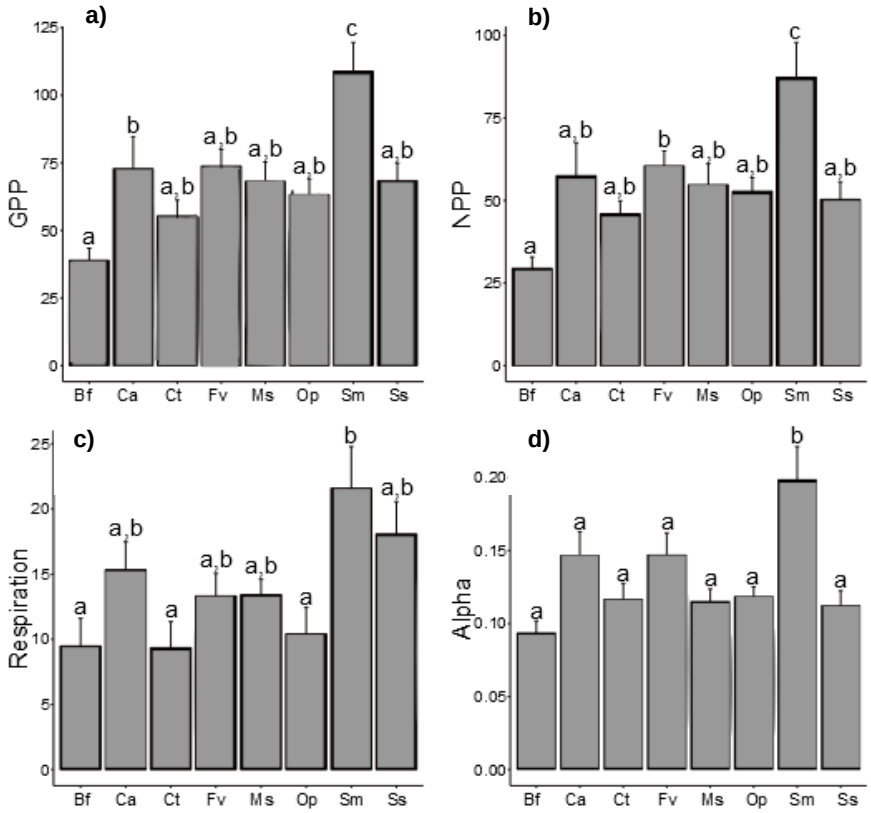

Figure 2.7.Mean (+SE, n=4) gross primary productivity (a), net primary productivity (b), respiration rate (c), and alpha (d) for single species assemblages for the eight seaweed used in our experiment. (S.s. *Halopteris scoparia, B.b. Bifurcaria bifurcata, C.t. Chondracanthus teedei, O.p, Osmundea pinnatifida, C.a. Chondracanthus acicularis, M.s. Mastocarpus stellatus,* , F.v. *Fucus vesiculosus*, S.m. *Sargassum muticum*). All measures refer to the whole assemblage. Bars sharing same letters did not differ in *a posteriori* SNK test at p-level 0.05

2.4.1.1. Functional performance of multiple species assemblages: Richness, Evenness and Identity effects

Functional performance of the macroalgal assemblages revealed some significant effects. The consistent effect of the seaweed identities on the performance of the assemblages was proved by the significant effect of identity for all the functional proxies measured (Table 2.1). Additionally, the interaction Richness x Evenness was significant for GPP and marginally not significant (0.10 >p > 0.05) for the other three functional proxies. This interaction resulted from a positive effect of evenness on gross productivity at low richness treatments but no effects on those assemblages with higher species richness (Figure 2.8). Similar patterns were found for NPP, Respiration and Alpha.

Table2.1. Summary of ANOVA analyses of the effects of Species richness, Evenness and Identity on the gross primary production (GPP), net primary production (NPP), respiration rates (RESP) and photosynthetic efficiency at low intensity levels (ALPHA). Numbers in bold indicate significant effects

		GPP		NPP		RESP		ALPHA	
Source	**df**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**P**
SR	1	0.09	0.770	0.06	0.816	1.24	0.3088	0.05	0.82
Ev	1	2.16	0.191	1.54	0.273	0.41	0.5441	1.11	0.33
Id (SR)	6	4.79	**<0.001**	4.44	**0.002**	4.55	0.001	**3.31**	**0.01**
SRxEv	1	7.09	**0.03**	5.82	0.052	8.77	0.0253	5.93	0.05
EvxId(SR)	6	1.52	0.192	1.73	0.135	1.53	0.1888	1.97	0.08

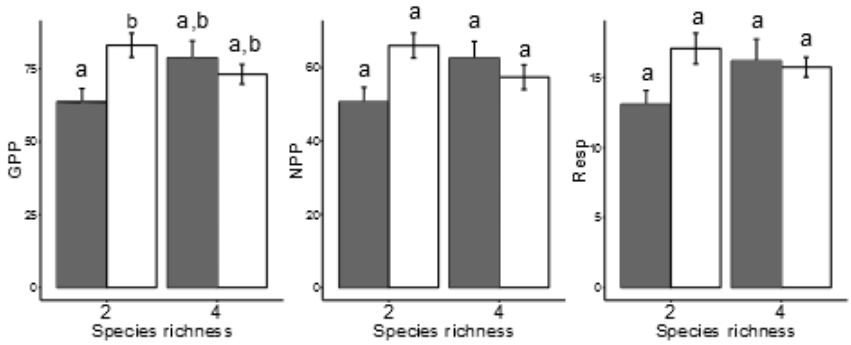

Figure 2.8.Mean (+SE, n=16) values of gross primary productivity (GPP), net primary productivity (NPP) and respiration rate (RESP) for each richness and evenness treatment. Filled bars correspond to low evenness treatments, white bars to high evenness treatments. Bars sharing same letters did not differ in *a posteriori* SNK test at p-level 0.05.

2.4.1.2. Functional performance of multiple species assemblages: Overyielding

LRR ratios of gross primary productivity (GPP) and net primary productivity (NPP) were significantly affected by the interaction between species richness and evenness (SRxEv) and by the identity (Id) predictors (Table 2.2). The interactions SRxEv in terms of the overyielding ratios suggested that the effect of evenness vanished with diversity. Thus at low diversity, higher evenness improved the performance of the assemblages relative to their expected performance from monocultures (Figure 2.9), but in assemblages with four species the effect was negligible. It is worthy to note than average LRR at high diversity were positive suggesting an better productivity than expected from monocultures.

Table2.2. . Summary of ANOVA analyses of the effects of Species richness, Evenness and Identity on the LRR ratios for the gross primary production, (GPP) net primary production, (NPP) and respiration rates (RESP) after 15 days of the experiment. Numbers in bold indicate significant effects.

		LRR GPP		LRR NPP		LRR RESP	
Source	**df**	**F**	**p**	**F**	**p**	**F**	**p**
SR	1	0.03	0.8644	0.02	0.8937	0.04	0.848
Ev	1	12.36	**0.0126**	6.83	**0.04**	2.31	0.5179
Id (SR)	6	7.23	**<0.001**	6.11	**<0.001**	6.79	**<0.001**
SRxEv	1	34.28	**0.0011**	24.92	**0.0025**	2.20	0.1884
EvxId(SR)	6	0.31	0.9273	0.43	0.8556	1.45	0.2165

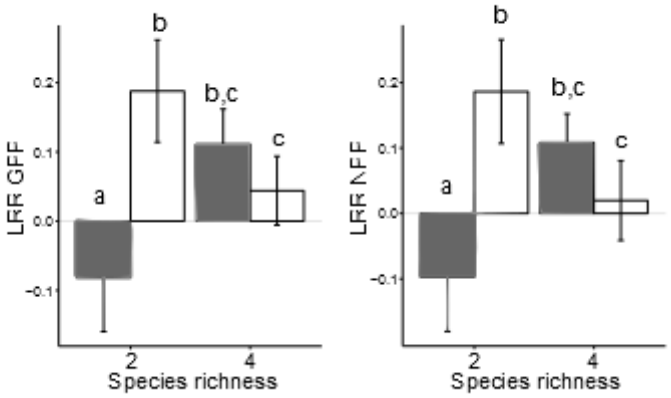

Figure 2.9.Mean (+SE, n=16) values of Log Response Ratios for gross primary productivity (LRR GPP) and net primary productivity (LRR NPP) for each richness and evenness treatment. Filled bars correspond to low evenness treatments, white bars to high evenness treatments. Bars sharing same letters did not differ in a posteriori SNK test at p-level 0.05.

2.4.1.3. Functional performance of multiple species assemblages: pairwise relationships between continuous metrics

Pairwise analyses showed that neither species richness nor evenness were significant predictors of assemblage's productivity-related responses or overyielding (LRR). The same was true for the two functional diversity indexes examined (FD & FDis). Conversely, community mean traits did explain significant proportions of variation in community biomass production than functional trait diversity support the importance of species traits effects. Thus, assemblages with larger CWM.SLA, i.e. larger overall specific leaf area were more productive. Conversely, assemblages with overall thicker fronds (larger CWM.LT were less productive (Table 2.3). In the case of LRR (overyielding indicator), the significant community mean traits predictors were CWM.LDMC (leaf dry mass content) with a positive effect and CWM.VS with a negative effect on the overyielding indexes (Table 2.4).

Furthermore the presence of certain species seemed to have significant effects on the performance of the assemblages. Particularly intense were the negative effects *Bifurcaria bifurcata* and the positive effects of *Chondracanthus teedei* and *Sargassum muticum* on the productivity of the assemblages (Table 2.3). *Sargassum muticum* effect reversed in the case of overyielding suggesting negative interactions with the other species. The same occurred with *Fucus serratus* (Table 2.4).

Table2.3. Slope parameter estimations of the pairwise relationships (OLS) between Species richness, evenness, Functional diversity (FD & FDis), community-weighted mean trait and abundance of the different species with the different proxies of assemblages functioning used in our study (GPP, NPP, Respiration and Alpha). Positive and negative symbols reflect the sign of the effect. *P < 0.05; **P <0.01; ***P < 0.001. Significant predictors in bold.

Single predictor linear models

PREDICTORS	GPP	NPP	RESP	ALPHA
Species Richness	1.27 n.s.	0.83 n.s.	-0.44 n.s.	0.001 n.s.
Evenness	29.09 n.s.	21.95 n.s.	-7.14 n.s.	0.04 n.s.
FD	-5.81 n.s.	-4.89 n.s.	0.99 n.s.	-0.008 n.s.
FDis	-4.33 n.s.	-3.15 n.s.	-1.18 n.s.	-0.005 n.s.
CWM.SLA	**29.05 ****	**21.48 ***	**7.56 ****	0.03 n.s.
CWM.LDMC	2.01 n.s.	3.25 n.s.	-1.11 n.s.	0.006 n.s.
CWM.LT	**-40.1 ****	**-30.6 ***	**-9,58 ****	**-0.05 ***
CWM.VS	7.36 n.s.	8.36 n.s.	-1.00 n.s.	0.01 n.s.
H. scoparia	-0.2 n.s.	-0.15 n.s.	-0.04 n.s.	0.0008 n.s.
B. bifurcata	**-0.31 *****	**-0.25 ****	**-0.06 ****	0.0008 n.s.
C. teedei	**0.42 ****	**0.33 ***	**0.09 ***	**0.006 ***
O. pinnatifida	0.18 n.s.	0.12 n.s.	**0.05 ***	0.0002 n.s.
C. acicularis	-0.07 n.s.	-0.06 n.s.	-0.008 n.s.	0.00006 n.s.
M. stellatus	-0.18 n.s.	-0.15 n.s.	-0.008 n.s.	-0.0003 n.s.
F. serratus	0.13 n.s.	0.11 n.s.	0.02 n.s.	0.0002 n.s.
S. muticum	**0.24 ****	**0.22 ****	0.02 n.s.	**0.0004 ***

Table2.4.Slope parameter estimations of the pairwise relationships (OLS) between Species richness, evenness, Functional diversity (FD & FDis), community-weighted mean trait and abundance of the different species with the LRR for the productivity proxies GPP, NPP and Respiration). Positive and negative symbols reflect the sign of the effect. *P < 0.05; **P <0.01; ***P < 0.001. Significant predictors in bold.

Single predictor linear models

PREDICTORS	LRR GPP	LRR NPP	LRR Resp
Species Richness	0.028 n.s.	0.009 n.s.	-0.01 n.s.
Evenness	0.39 n.s.	0.38 n.s.	-0.48 n.s.
FD	0.03 n.s.	0.07 n.s.	0.13 n.s.
FDis	0.06 n.s.	0.09 n.s.	0.10 n.s.
CW.SLA	-0.02 n.s.	-0.16 n.s.	**-0.59 ****
CWM.LDMC	**-0.40****	**-0.34 ***	**0.66 ****
CWM.LT	0.19 n.s.	0.33 n.s.	0.47 n.s.
CWM.VS	**-0.39 ****	**-0.33 ***	**0.7*****
H. scoparia	0.002 n.s.	0.004 n.s.	0.003 n.s.
B. bifurcata	0.002 n.s.	0.003 *	0.002 n.s.
C. teedei	**0.008 *****	**0.007 ****	**-0.013 ****
O. pinnatifida	0.002 n.s.	0.001 n.s.	-0.006 ***
C. acicularis	-0.001 n.s.	-0.001 n.s.	-0.0005 n.s.
M. stellatus	-0.001 n.s.	-0.001 n.s.	0.0008 n.s.
F. serratus	-0.005 n.s.	**-0.005 ***	**0.006 ***
S. muticum	-0.004 n.s.	**-0.003 ****	**0.004 ****

2.5. Discussion

In our experimental study we examined how several diversity-related traits affected the functional performance of synthetic macroalgal assemblages, using primary productivity as a proxy for ecosystem function. We used an experimental design derived from Benedetti-Cecchi (2004) to separate the influence of species richness, evenness and identity. Additionally we calculated indexes of functional diversity and community mean traits and examined their ability as continuous predictors to estimate the productivity and overyielding indexes in the assemblages.

Our findings showed the prevalence of species traits, i.e. identity effects, as the main driver of the performance of the assemblages. Species traits effects were apparent in the analyses of variance where the identity factor was significant for all the analyses performed. They were also proved by the consistency as significant predictors in the linear models of some community-weighted mean functional traits and species biomass. Furthermore and despite the limitations of this type of short manipulative experiments, i.e. artificial assemblages, short in duration and small in spatial scale (Gamfeldt et al., 2015), we found that the effects of species traits were not always additive and species interactions modified partially their functional performance when coexisting in a community. Thus, relative species abundance (evenness) promoted productivity on low diversity treatments but no on high diversity treatments.

The confirmation that species and community mean traits explained a larger proportion of variation in community productivity than species and functional diversity supports the general consensus that ecosystem processes are governed by functional traits of species (i.e. identity) and dominance effects (Roscher et al., 2012; Enquist et al., 2015). Hence, the results are largely in agreement with Grime's Mass Ratio Hypothesis or MRH. The MRH states that ecosystem functioning is determined by the characteristics or traits of the dominant (largest biomass) species (Grime, 1998). Strong species traits effects are a frequent outcome of biodiversity-functioning experiments, particularly those performed at small local-temporal scales (Cardinale et al., 2006).

Previous research on seaweed assemblages found also strong species-specific traits effects (Bruno et al., 2005; Bruno et al., 2006; Griffin et al., 2009). In our study for example, assemblages including the brown seaweed *Sargassum muticum* generally

showed high productivity and respiration ratios with increasing effects at higher biomasses. The effects of this species was opposite regarding the LRR ratio for two of the productivity proxies, suggesting the existence of negative interactions with other species. Interestingly, *Sargassum muticum* is a Japanese invasive species in the European Atlantic shores and in certain areas the species displaced the very abundant native *Bifurcaria bifurcata*, modifying severely primary productivity patterns in invaded areas (Vaz-Pinto et al., 2014). Not surprisingly *Bifurcaria bifurcata* was the species with lower overall performance in our experience. Thus our assemblages seems to mimics patterns that are already described in other studies from natural systems. Species traits or identity effects also have been identified as very relevant when considering other community level processes in macroalgal assemblages like resistance to invasion (Arenas et al. 2006, Vaz-Pinto et al., 2014).

Besides the preponderance of traits or identity effects, our experiment revealed the existence of other diversity-related effects. Thus, we found a positive effect of evenness on the productivity of assemblages at low diversity treatment, effect absent at high diversity treatments. Positive effects of evenness on primary production on terrestrial system have been recorded in several experimental studies (Stevens & Carson, 2001; Kirwan et al., 2007), although dominance by large species may alter this effect (Mulder et al., 2004; Orwin et al., 2014). In marine studies, evenness has also being suggested as a potential mechanism promoting primary productivity on natural seaweed communities (Arenas et al., 2009), however our study is among the first to examine simultaneously and experimentally species richness and evenness on seaweed assemblages (see also Lemieux & Cusson, 2014). The mechanisms behind the positive effect of evenness could be the enhancement of resource use complementarity with increasing evenness, boosting ecosystem performance (Polley et al., 2007) and generating positive overyielding indices. The intensity of evenness effect could be stronger at low diversity treatments because when the assemblages are built with few species, the dominance of one single species may reduce largely the relevance of potential complementary interactions among the few remaining non-dominant species.

We did not find strong evidences supporting the positive effects of richness, but obviously it should not be rule out as a driver. Our experiment did not use a large richness gradient and on overall multiplying by two the number of species in the assemblages, i.e. from 2 to 4 spp, only increased around 3 % GPP (average GGP

2spp= 73.41±3.48 mg O_2 h^{-1}, 4 spp= 75.95±3.36 mg O_2 h^{-1}, n=32). This effect seems far from the estimated effect of richness found in literature. On terrestrial systems, Hooper et al., (2012) estimated that a fifth percent species loss would mean a reduction of biomass production around 13 %. If we consider also assemblages with only one functional group, our results were closer to this study. Thus, including single species assemblages, the average GPP in monospecific assemblages was 68.69±4.11 mg O_2 h^{-1}, i.e. a value almost 10 % lower than the high diverse assemblage. Bruno (2005) found that relative yields in multispecies assemblages of seaweeds where 13 % greater than monocultures but results were not significant. However, these type of short term experiments frequently do not find any species richness effect (Cardinale et al., 2007; van Ruijven & Berendse, 2005). Studies manipulating macroalgal richness have been often unsuccessful finding obvious richness effects. Some other experiments have also found positive effects of species richness on the performance of macroalgal assemblages, irrespective of the response measured. Middelboe & Binzer, (2004) and Bruno (2006) found positive effects of richness on macroalgal photosynthetic rates. Bruno et al (2005; 2006) reported higher biomass accumulation in richer assemblages and Bracken & Stachowicz (2006) noticed positive richness effects on community nutrient uptake. However, whenever the experimental design allowed disentangling the mechanisms behind the positive relationship, species traits, i.e. identity effects were identified as the dominant effect (Bruno et al., 2006).

The approach used in this study allowed us to create the experimental treatments required to examine direct additive and synergistic effects of species richness, evenness and community traits on the productivity of marine macroalgae assemblages. Those direct and interactive effects are almost impossible to examine on natural assemblages. Furthermore observational studies cannot establish causality among drivers and effects. This type of experimental approaches generally lack of strict realism, they do provide clean tests of specific predictions Our approach was able to detect some of the interactive effects of species richness and evenness suggested in literature. However, the intensity of these effects were subtle and were overridden by the strong effects of species functional traits.

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Part III

Chapter 3

The effects of physical disturbance on the relationships between diversity and productivity on natural seaweed communities

The effects of physical disturbance on the relationships between diversity and productivity on natural seaweed communities

Aquatic Ecology & Evolution Group, Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

Coastal Biodiversity Group, Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

The effects of physical disturbance on the relationships between diversity and productivity on natural seaweed communities. *In prep*.

Abstract

Natural and human-induced disturbance have an effect on the structure and functioning of natural systems. Ecologists have recently begun to explore how forces that modify community structure might simultaneously modify the effects of species richness on ecological processes. On intertidal communities disturbances over a range of scales and intensities are frequent, often causing partial removal of dominant species. However whether these resources release promotes diversity and alters assemblages functioning it is largely unknown. In this study we aimed to generated diversity gradients using disturbance gradients and measured the relationship between diversity and productivity. Two experiments were conducted where physical disturbance was applied onto natural rockpools assemblages and structural and functional responses were measured “in situ”. Results showed contrasting effects, both structural and functionally, on the two experiments. In one experiment, the structural changes created by the disturbance did not result on functional changes on the assemblages. In the second experiment no structural changes were detected but gross primary productivity was higher in perturbed plots than in controls. Hence our results suggest some uncoupling at these local scales in the relationship between structure and functioning in these type of assemblages.

Keywords*: Disturbance, Diversity-productivity coupling, Rockpools.*

3.1. Introduction

It is well established that biodiversity promotes a full range of ecosystem functions with large ecological relevance, such as biomass production, decomposition, biogeochemical cycling and ecosystem stability (Hooper et al., 2005; Cardinale et al., 2012, Hooper et al., 2012). Biodiversity also benefits ecosystem goods and services that nourish directly and indirectly from natural ecosystems into the socio-economic systems (Costanza et al., 1997, 2007, Daily et al., 2000; Cardinale et al., 2012). In the last decades, large scale and persistent human disturbances are having major negative impacts on natural systems altering community structure, damaging communities and threating the maintenance of very relevant ecosystem processes but also life-supporting ecosystem goods and services (Cardinale et al., 2012, Hooper et al., 2012). However disturbances are not exclusive result of human activities. Natural systems suffer the effects of natural disturbances regularly. In fact disturbance is a major driver of communities' dynamic with a relevance similar to competition and predation (Sousa, 1984).

Disturbance is often defined as "any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability or the physical environment" (White & Pickett, 1985). Disturbances are particularly relevant on coastal benthic systems, where space is the first limiting resource (Paine & Levin, 1981). In these systems, natural disturbance plays an important role in the species turnover and the maintenance of highly diverse assemblages (Menge,2000). By releasing resources and creating habitat heterogeneity, disturbances can maintain, or even increase species diversity, however these effects are strongly scale-dependent. Nowadays, human-induced disturbances of increasing intensity and frequency threat ecosystems resilience and thus the functions ecosystems provide. Last decade, ecologists begun to explore how forces that modify community structure might simultaneously modify the effects of species richness on ecological processes. Disturbances that alter the structure of communities, changing the number of species and abundance patterns, are likely to impact ecosystem functions directly by changing overall assemblage traits and species interaction balance and potentially mediate in the relation between species richness and ecosystem functioning relationship (Cardinale & Palmer, 2002).

The degree to which a disturbance affects ecosystem functioning depends greatly on the sequence of species lost and which biological traits were affected (Cardinale et al., 2012). If diversity may promote ecosystem functioning and some disturbance regimes may promote diversity, we could expect a positive effect of disturbance on the performance of the ecosystems. Except if these diversity-promoting processes did not prompt positive interactions like complementarity or facilitation among species, decoupling the relationship between diversity and ecosystem function properties (Mouquet et al., 2002).

Rockpools are very interesting systems to use for these kind of research. On these intertidal habitats, patchy macroalgal assemblages are maintained by different processes such as physical disturbance, successional transitions, predation, spatial heterogeneity, recruitment limitations, differential life histories, etc. (Lubchenco, 1982; Chapman, 1990; Benedetti-Cecchi & Cinelli, 1996; vanTamelen, 1996; Menge et al., 2005). In this study we examined the effects of disturbance on natural rock pool assemblages, but looking not just into the structural effects but also into the functional consequences of disturbances.

In our study we carried out two different experiments to examine the functional effects of perturbation on rockpools assemblages. In the first experiment we used the predictions derived from the intermediate disturbance hypothesis (IDH, Connell, 1978) to examine functional effects of diversity gradient created by disturbances. The IDH theory suggest that species diversity is maximized at intermediate disturbance levels where competitive and opportunistic species may coexists. This theory is contested by many authors (Wooton et al., 2009) but it was considered as a convenient framework to explore the potential functional impacts of disturbance on intertidal hard bottoms.

In a second experiment we examined diversity-productivity relations after the perturbation of two specific type of assemblages, characterized for a high dominance of single seaweed morpho-types. We use a disturbance experiment to examine the functional effects of increasing biodiversity by reducing the dominance of certain species.

Both experiments aimed ultimately to test the same hypothesis: In highly dynamic systems like intertidal areas where interspecific interactions are not always the key community structuring driver, physical disturbance in assemblages may alter the

structure of assemblages increasing diversity but do not necessarily improve the functional performance of the community.

Our experiments aimed to gain relevance for natural systems by using an experimental approach with natural assemblages and by measuring structural and functional responses "*in situ*", i.e. at the rockpools. Additionally, we used a disturbance procedure which mimics natural disturbances. We did not manipulated directly diversity but we tried to simulate a natural process which is reported to modified communities diversity. We used the most frequent type of natural disturbance, i.e., pulse disturbance which are short term, delineated disturbances, from which the system can return to its previous equilibrium (Bender et al., 1984). To perform the experiments we developed new in situ experimental chambers, which allowed to examine productivity irradiance relationships in natural assemblages.

3.2. Materials and Methods

3.2.1. Experimental site and design

As explained above, two experiments were carry out to test our initial hypothesis. Both experiments were performed at Praia de Moledo (Northern Portugal 41°50′22″ N, 8°52′30″ W) during the spring and summers of 2011 and 2012. Moledo is a typical rocky shore with a large and smooth intertidal granite platform and abundant species rich rockpools. This rocky shore is highly exposed to oceanic swells that are particularly intense during the winter. It has a semi-diurnal tidal regime, with the largest tidal range of 3.5–4 m during spring tides. Mean sea surface temperatures of the intertidal pools in this area are close to 15 ºC (Cacabelos et al., 2013).

Experiment 1

In spring 2011, four large mid-shore pools with similar physical conditions and assemblages were selected in Moledo. At each pool, 12 plots of 16x16 cm and fully covered by seaweeds were haphazardly selected and tagged in the corners with screws and labels. Plots were separated each other by a minimum distance of 50 cm to ensure some independence.

Three disturbance treatments were applied to the plots: control (i.e., no removal), low disturbance (i.e., 25 % of algal cover removed, hereafter LD), and high disturbance intensities (i.e., 50% of algal cover removed, hereafter HD).Treatment were randomly replicated in 4 plots per pool. In those plots assigned to the low and high disturbance treatment, algae were manually removed by carefully scrapping the seaweed from the base with a scrapper, and using as a guide two PVC templates perforated previously in a random pattern. The template allowed us to leave un-scrapped 25% of the plot (LD treatment) and 50% of the plot (HD treatment).

Thus the design of this first experiment included 2 factors: Disturbance, fixed factor with 3 levels (C, LD, and HD) and a random factor Pool with 4 levels. Both factors were orthogonal. Once the treatment was applied, we wait three months to allow new species to recruit and measured the changes in the structure and the functioning of the plots.

Species richness and relative abundance of each species was estimated using a non-destructive sampling procedure. Percentage cover estimates were obtained by dividing each quadrate into 25 sub-quadrates, assigning to each taxon a score from 0 (absence of that taxon) to 4 (a whole sub-quadrate covered by that taxon) and adding up the 25 estimates (Dethier et al., 1993).

Once the structural measures were done, we carry out “in situ” incubations to evaluate the community responses to the treatments. Due to the complexity and logistic constraints to perform these incubations, we only incubated 2 replicates per treatment (i.e. 24 plots were selected for incubations).

Experiment 2

In spring 2012, we selected very large patches of two of the most dominant morpho-functional groups in several large rockpools in Moledo. Those morpho-functional groups were: i) *Corallina* spp dominated turf and ii) *Bifurcaria bifurcata* dominated beds. Healthy, non-perturbed patches of these two morpho-functional assemblages are characterized frequently by a large dominance of the structural species and relative low diversity, although epiphytes may occasionally be very abundant on *Corallina spp* patches in summer. In each of these morpho-functional groups, we tagged eight plots of 256 cm^2 and applied a disturbance treatment on half of them. From our previous experience and with the idea of achieving a large effect we used only one disturbance treatment equivalent to the high disturbance treatment in experiment 1, i.e. removing 50 % of algal cover. Disturbance procedure was the same than in experiment 1. Thus, plots in the disturbance treatment were scraped down to bare rock so that no visible organisms remained; even crustose coralline algae were removed. (Figure3.1)

Structural and functional measures were carry out using the same procedure than in experiment 1, except that in experiment 2, all the biomass from the plots was collected once the functional measures were taken.

Figure 3.1.Picture of one of the plots with a disturbance treatment. In this case disturbed plot dominated by *Bifucaria bifurcata* and 50% of algal cover removed.

3.2.2 Experimental incubations

Primary production of natural macroalgal assemblages was examined *in situ* using custom-built incubation chambers fixed to the bottom of the experimental rock-pools. Chambers were designed to be secured around the assemblages of benthic intertidal macroalgae, without perturbing them. They were made of an acrylic tube (three heights of 30, 40 & 50 cm, to accommodate the varying pool depths), with a clear Perspex attachment quadrate base (see Fig. 3.2). Chambers were fixed to the experimental plots throughout a quadrate frame which was previously secured in the area of the plots to the bottom of the pool using screws. The Perspex tube had an internal diameter of 25 cm and but the area covered by the base was 256 cm^2, i.e. the same size than the experimental plots. Before chambers could be effectively fixed to the rock, the area around the targeted assemblage was cleared from algae and invertebrates. This was done by scraping the substratum to ensure that the entire surface was flat enough to allow a watertight seal. Base frames were made from 1 cm thick PVC where a 0.5 cm thick piece of rubber was glued. To avoid leakage problems, a 1 cm thick piece of closed-cell polystyrene foam was glued in the base filling any possible gaps. A similar procedure was used to seal the basal frame to the bottom of the pool.

When tightened to the rock, the foam compressed and filled slight surface irregularities, forming a good seal between the rock and the base plate. (Figura3.2)

Four long, threaded bolts were also used to attach the main chamber to the base frame. The seal between the chamber and the base frame was maintained using wing nuts that allowed quick fixing compress when the long bolts were tightened.

The volume of water contained within the chambers varied depending on the relative position of the chamber and the depth of the pool, this volume was estimated for each incubation.

Figure3.2. Base frame and chambers incubators.

Once the chambers were fixed, the water from each chamber corresponded to the water remaining in the pool during low tide. Chambers were covered with three dark glass meshes to achieve gradual increments of light and a black plastic to exclude light was used to simulate complete darkness. This process will allow us to examine the productivity irradiance relationship in the plots under the different experimental treatments (Figure 3.3 b)

During the experiment, water within the chambers was mixed using a battery-powered bilge pump. Water movement inside the incubation chambers was maintained through a submersible pump (10 L/m) equipped with diffusers to reduce turbulence and was

placed in the chamber was stirred in a circular vortex motion. It was powered by 12-Volt for supply voltage battery housed in an outside case.

Average daily seawater temperature was 14.5 ºC (±0.07, SE) was recorded with a data-logger (Hach® HQ40). Productivity-irradiance (P-I) curves were generated using five levels of irradiance 0, 19%, 31%, 52% and 100% of incident light. Irradiance can be extremely variable in natural conditions in the field. During the incubations, incident irradiance in the area was measured continuously using a scalar quantum sensor (Biospherical® QSL-2000 143 Radiometer) connected to a computer.

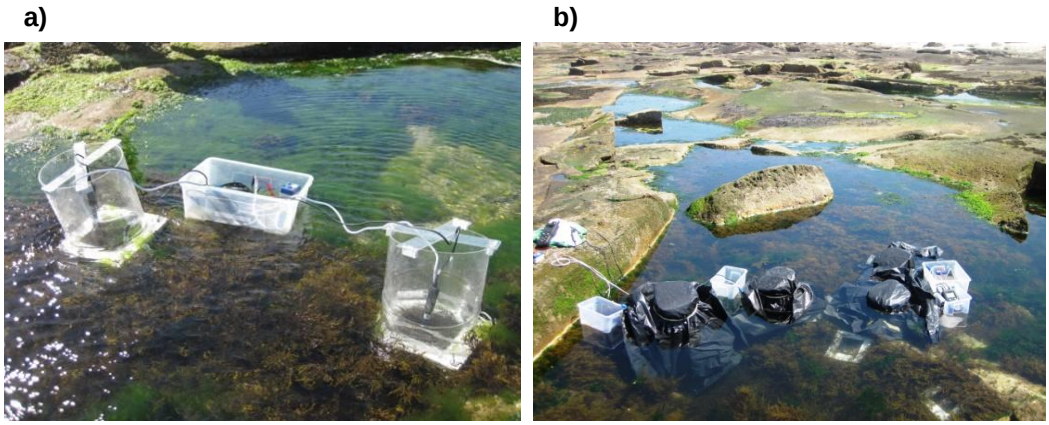

Figure 3.3. Procedure of in situ incubation.a) Light period and b) dark period.

3.2.3 Ecosystem functioning surrogates

Dissolved oxygen concentration within each chamber was measured using a luminescent dissolved oxygen probe connected to a data-logger (Hach® HQ40) that registered oxygen concentration and temperature every 30 seconds, and was continuously monitored. For each P-I incubation, the successive irradiance periods lasted between 20 and 25 min, and the whole set of incubations took around 2:30-2:45 hours per plot.

All the experimental plots included a variety of sessile and mobile invertebrates. To avoid respiration interferences by these creatures, all visible invertebrates were removed from the target area. However, removing all the invertebrates from *in situ* assemblages was often impossible because of their small size (Tait & Schiel,2011). Respiration was measured by covering the chambers with a layer of black plastic.

Following periods of photosynthesis, the relative respiration rate of macroalgae can be substantially elevated, known as photorespiration (Reiskind et al., 1989). Therefore, once the chambers were covered they were allowed to settle for 30 minutes before respiration measurements were started to limit the photorespiration effect.

Respiration and productivity were estimated through oxygen fluxes by regressing oxygen concentration (µmol) through time (s^{-1}) during dark and light periods of increasing intensities. Estimations were normalized by biomass (second experiment) and corrected by seawater volume inside the chamber to take into account the different volumes of the incubations.

Four ecosystem functioning surrogates were determined per plots: (Arenas et al. 2009) 1) maximum net primary productivity (max NPP), maximum productivity (i.e. maximum slope for the oxygen concentration over time) recorded at any light intensity (mg O_2 h^{-1}); 2) Gross primary productivity (GPP); (Arenas et al., 2009) 3) assemblage respiration; i.e., the oxygen consumption during the dark period of the incubation (mg O_2 h^{-1}); and (Arenas et al. 2009) 4) photosynthetic efficiency at low irradiance (α), estimated using ordinary least squares (OLS) regressions for the light-limited portion of the curve (mg O_2 $\mu Em^{-2}s^{-1}$).

3.3. Statistical analyses

To examine structural changes in the assemblages in relation to the disturbance treatments of the experiments, we used multivariate permutation analysis of variance, Permanova), Anderson 2001. In the case of experiment 1, Permanova included two factors: i) Disturbance treatment, a fixed term in the analysis with three levels, undisturbed plots= Control (C), Low disturbed plots (LD) and High disturbed plots (HD) and ii) Pool a random factor with four levels and crossed with the previous factor. In experiment 2 Permanova test included also a fixed term disturbance treatment this time with two levels Control (C), and disturbed plots (D) and random community identity factor with two levels also (*Corallina* and *Bifurcaria*). We used SIMPER analysis performed by Primer 6 for Windows to identify which seaweed species contribute to the dissimilarity between the perturbation treatments.

Structural univariate responses such as species richness and evenness were examined using standard analysis of variance using the same designs as in the Permanova analyses.

Functional responses (NPP, Respiration and alpha) were first investigated using analyses of variance as in the case of structural univariate designs. In the case of experiment 1, the design was slightly modified removing the random factor pools because the reduce number of replicas. Also a linear model was used to identify relationships between continuous structural variables like species richness and evenness and the functional variables. Our linear models also include disturbance as a categorical predictor as a categorical predictor. All these analyses were done in R-software Core Team (2014).

3.4. Results

3.4.1. Experiment1

3.4.1.1. Experiment1. Structural measures

Table 3.1. Multivariate permutation analysis of the structural data (i.e. species cover) of the experiment 1. In bold those predictors with significant effect

Source	df	SS	MS	Pseudo-F	P(perm)
Treat	2	6615.5	3307.7	6.8695	**0.004****
Pool	3	3220.8	1073.6	3.7165	**0.002****
Treat x Pool	6	2889.1	481.51	1.6669	0.085
Res	36	10399	288.87		

Multivariate permutation analysis (Permanova) of the data from experiment 1 showed that our disturbance treatments clearly modified the assemblages (Permanova, $p<0.01$). Analysis also revealed the existence of differences in assemblages between pools. No interaction between disturbance treatment and pool factor was found suggesting than the effects of the disturbance were relatively consistent across pools (Table 3.1). Permutation-based pairwise tests among the three treatments found structural differences between the control assemblages and the other two disturbance treatments, i.e. C≠LD=HD.

SIMPER analyses performed to identify the species that contributed most to dissimilarities between treatments, revealed that changes among treatments were mainly due to a general reduction in dominant species like *Corallina elongata*, *B. bifurcata* and increases on the cover of species like *Lithophyllum incrustans* (Table 3.2).

Table3.2. Multivariate Simper analyses to select the species which make the highest difference among the disturbance treatments used in experiment 1. Analyses carried out with the untransformed cover data.

Species	Control % Cover	Low D % Cover	High D % Cover	Control-LD Average Dissimilarity	Control-HD Average Dissimilarity
Corallina elongata.	40.69	32.88	21.38	19.2	22.6
Lithophyllum inc.	4.19	16.56	21.44	11.5	15.4
Ceramium spp.	15.88	16.50	10.63	10.7	11.4
Bifurcaria b.	7.31	0.50	1.56	6.0	7.2

Despite these changes on the structure of the assemblages, we did not find any effect of the disturbance on descriptors like species richness or evenness (Pielou J' index), Anova, $F_{2,6}$: 3.17, $p>0.05$ and $F_{2,6}$: 0.79, $p>0.05$ for species richness and evenness respectively. Thus our results did not support the IDH in these type of habitats.

3.4.1.2. Experiment 1. Functional measures

Anova analyses did not find any effect of disturbance on the primary productivity proxies examined. Neither, maximum primary productivity, respiration nor photosynthetic efficiency at low light (alpha) were modified by the treatments of disturbance (Anova, $p>0.05$ in all the cases). Similarly, when we examined the functioning proxies using linear models which included as continuous predictors, species richness and evenness and as a categorical predictor, disturbance treatment did not find any significant effect (lm, $p<0.05$ for all the independent variables) (Figure 3.4). Thus in this field experiment, structural and functional responses to perturbation in the pools seemed to be uncoupled.

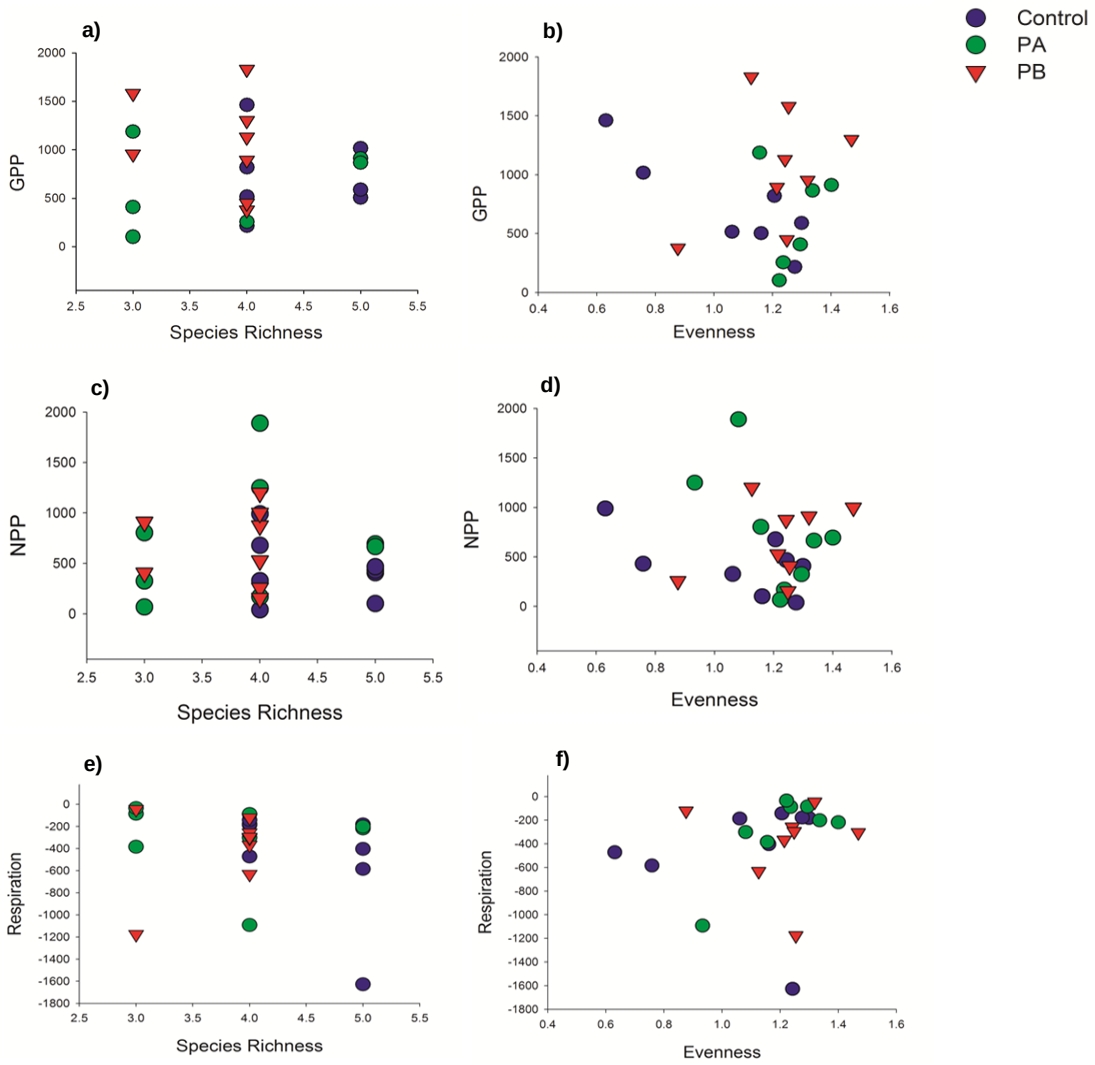

Figure 3 4 Relationships between species richness and evenness with the functional proxies measured in experiment 1:a)maximum gross primary productivity(GPP) and species richness; b) maximum gross primary productivity(GPP) and evenness c) maximum net primary productivity (max NPP) and species richness; d) maximum net primary productivity (max NPP) and evenness; e) Respiration and species richness; f) Respiration and evenness. Blue circles: Control - No disturbed plots; Green circles: Mid disturbance treatment; Red triangles: High disturbance treatment.

3.4.2. Experiment 2

3.4.2.1. Experiment 2. Structural measures

Permanova analysis using Bray-Curtis dissimilarity index on four root transformed data of biomass did not find any effects of disturbance on the assemblages, or on its interaction with community type. Community type itself was a significant factor (Permanova, $p<0.01$, Table 3.3). Thus unexpectedly our perturbations did not have the anticipated effect on those two types of communities (*Bifurcaria* and *Corallina* dominated patches). Anova analyses did not find any effect of the disturbance on the species richness, evenness or on the biomass of the assemblages (Anova $F_{1,1}$: 36.0, $p>0.1$; $F_{1,1}$: 0.49, $p>0.5$; $F_{1,1}$: 0.84, $p>0.5$, respectively). Interactions Treatment x Community were not significant either. Thus three months after the disturbance, the assemblages had already mostly recover from perturbation, at least regarding these structural parameters.

Table3.3.Multivariate permutational analysis of the structural data of the experiment 2.

Source	df	SS	MS	Pseudo-F	P(perm)
Treat	1	383.62	383.62	1.3611	0.232
Community	1	3666.5	3666.5	13.009	**0.001****
Treat x Com	1	234.72	234.72	0.910	0.438
Res	12	3382.1	281.84		

3.3.2.2. Experiment 2. Functional measures

Regarding functional proxies, and despite the lack of structural differences among the treatments, GPP of the assemblages was higher on disturbed assemblages than on control plots (Figure 3.6). NPP was marginally not significant and no differences were found for respiration or alpha (Table 3.3; 3.4) (Figure 3.5).

Table3.4.Multivariate permutational analysis data of the functional responses of the experiment 2.

		GPP			NPP		
Source	**DF**	**MS**	**F**	**P**	**MS**	**F**	**P**
Community	1	4.84	0.11	0.7467	22.00	1.28	0.2798
Disturbance	1	91.79	307.39	**0.0363***	38.77	48.10	0.0912.
Com x Dist	1	0.30	0.01	0.9359	0.81	0.05	0.8321
RES	12	44.27			17.17		

		RESP			ALPHA		
Source	**DF**	**MS**	**F**	**P**	**MS**	**F**	**P**
Community	1	47.47	2.66	0.1287	3.20	2.30	0.1555
Disturbance	1	11.25	91.15	0.0664	0.09	0.03	0.8866
Com x Dist	1	0.12	0.01	0.9351	2.85	2.05	0.1781
RES	12	17.83			1.39		

Finally as in the case of experiment 1, we did not found any relationship between species richness or evenness with any of the primary productivity proxies measured.

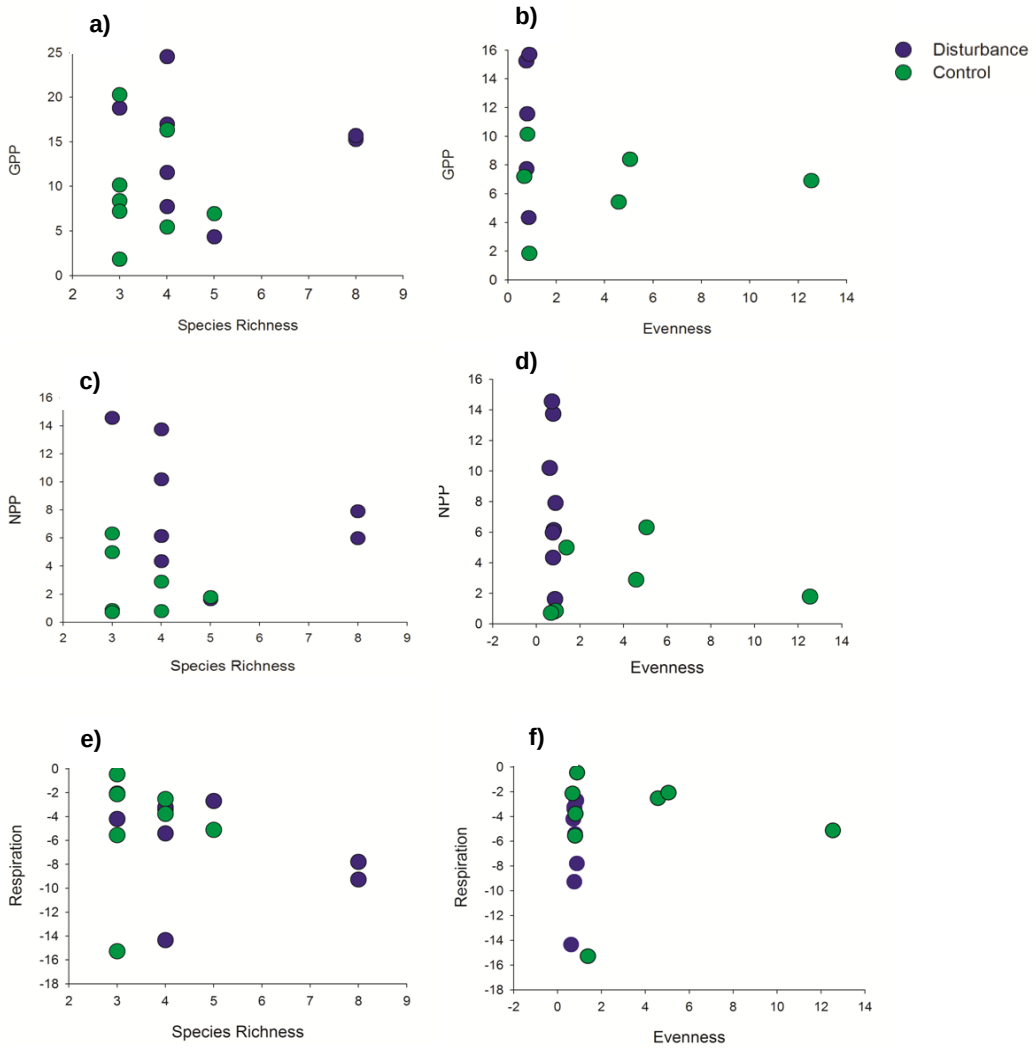

Figure3.5. Relationships between species richness and evenness with the functional proxies measured in this experiment 2: a) maximum gross primary productivity(GPP) and species richness; b) maximum gross primary productivity(GPP) and evenness c) maximum net primary productivity (max NPP) and species richness; d) maximum net primary productivity (max NPP) and evenness; e) Respiration and species richness; f) respiration and evenness. Blue circles: Disturbance, Green circles: Control.

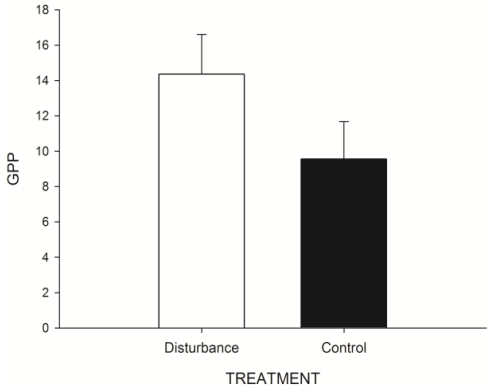

Figure 3.6 Relationships between gross productivity primary (GPP) with different treatment.

3.5. Discussion

Studies on biodiversity-ecosystem functioning in marine systems have progressed greatly in the last years (see review by Stachowicz et al., 2007;Gamfeldt et al., 2015). Like in terrestrial systems the current evidences point toward a direct link between diversity and ecosystem functioning, hence loosing diversity will reduce average ecosystem performance. Most of the research done on marine systems have been performed using synthetic assemblages, under very homogeneous and controlled conditions. How relevant are these results to natural assemblages where species are lost in a non-random way is still an open debate.

Physical disturbance by removing organisms, diminishing competition and releasing resources may facilitate the colonization of communities by new species. Thus disturbance may be a diversity-promoting processes. However the effect of disturbance on diversity is not always simple. The intermediate disturbance hypothesis (IDH) predicts that local species diversity is maximized at an intermediate level of disturbance (Grime, 1973; Connell, 1978). Basically the idea is that at low disturbance few species are present due to mechanisms of competitive exclusion. At intermediate levels of disturbance species diversity is maxima because both, good colonizers and good competitors, can co-exist. Finally at high disturbance few stress-tolerant species are present or opportunist species.

In our first experimental study we aimed to create some diversity gradient using the framework of the IDH hypotheses and test how this diversity gradient could relate with systems productivity. On those plot disturbed, the structure of the experimental assemblages changed because the relative abundance of species was modified, however no effects were found on the overall species richness or evenness. Furthermore structural changes were in this experiment disconnected with functional effects.

Highly disturbed communities like those inhabiting rockpools of very exposed shores may be highly functionally resilient. Opportunistic highly productive species may compensate quickly the reduction of productivity due to the loss of biomass of larger competitive species resulting from the perturbation. A similar result was found by (Martins et al., 2007) on rock-pools in South England. This author found no differences in productivity among pools recently disturbed (up to 5 months) and mature control

pools. A similar result was found by Crowe (Crowe et al., 2013) in a large scale experiment across European shores.

It is worthy to note that the type of disturbance applied in this type of experiments is probably very relevant in the functional response of communities. Our physical perturbation removed biomass and liberated resources which were soon claimed by the remained individuals. However other type of physical stressor, like pollution or temperature, by reducing the performance of most of the species would potentially have a very different effect on the system.

In our second experiment, we examined the productivity after the perturbation of two specific type of assemblages, characterized for a high dominance of single seaweed morpho-types, *Bifurcaria bifurcata* and *Corallina elongata* dominated assemblages. In this case and despite the fact that we applied the largest perturbation treatment we did not find any significant impact on the assemblages after three months, ratifying the results from the previous experiment and suggesting that rapid compensatory growth on these assemblages are able to restore the impact of physical disturbances in three months.

Nevertheless and despite the lack of a distinct structural change in the assemblages we found functional effects with increases on GPP of the community in this experiment. Probably our selection of target assemblages is very much related with this result. Those morpho-functional types are primary space holders with quite low primary productivity rates (see Chapter 2 and 5 of this thesis), thus increases of biomass of any companion species due to the release of space and other resources via physical disturbance may have strong consequences on the overall community assemblages.

Idiosyncratic and context dependent results are very common on biodiversity-functioning literature and deserve a closer attention to look for the underlying mechanisms generating these variable responses (Emerson et al., 2001).

We did not find any statistical relationships between species richness, evenness and the primary productivity proxies for any of the two experiments. This lack of relationship could result from the narrow range of species richness found in our experiment with only 8 species in the most diverse assemblages. In the past some authors claimed that small diversity gradients could be behind the lack of relationships found in some BEF research (Gamfeldt et al., 2015). We did not manipulated directly species richness. We used natural gradients, although the relatively small size of the experimental plots

resulted in this reduced gradient. In seaweeds and due to the small size of most seaweed species, interactions among individuals like those generating diversity effects occur at small scales similar to those used at the experiment. Thus it is unlikely that the reduced diversity gradient is preventing the occurrence of a relationship.
In our opinion it is probable that the high variability in the diversity-productivity relationships in this type of communities and the relative low relevance of species richness in determining the magnitude of assemblage's productivity is causing this lack of significant relationships. In fact, the identity of the species, or more precisely their functional traits, drove the productivity of the assemblages in other studies done in similar assemblages (Arenas et al., 2009; Griffin et al., 2009).

Finally it is also relevant to highlight that the experiments done in this chapter were carried out at the field and thus they were realistic. The experimental procedure, derived from other similar approaches (Migne et al., 2002), allows precise measures of functional parameters and thus has strong potential to be used on ecosystem functioning research on intertidal marine assemblages. However the logistic difficulties to carry out in situ P-I curves necessarily reduced the size of our experiment and limited the generalisation of the results.

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Part IV

Chapter 4

The functional consequences of succession: experimental studies using seaweed assemblages

The functional consequences of succession: experimental studies using seaweed assemblages

Aquatic Ecology & Evolution Group, Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

Coastal Biodiversity Group, Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

The functional consequences of succession: experimental studies using seaweed assemblages. *In prep*.

Abstract

Experimental studies in natural communities have created some controversy about the commonly accepted positive relationship between diversity and productivity. Ecological succession consists of the sequence of changes in community structure that occurs after a site has been disturbed. During ecological succession, mathematical models have demonstrated that the different coexistence mechanisms driving the successional dynamic can shape this relationship, generating both positive or negative relationships or even not relationship at all. macroalgal communities and rocky pools are very suitable systems to identify and evaluate these coexistence mechanism because succession is a relatively quick process compared with the terrestrial ecosystems.The objective of this chapter is to explore how successional dynamics modifies the relationships between diversity and ecosystem functioning. In this study two different scenarios are included: primary and secondary succession. Our results found in the case of primary succession no effects of diversity, evenness or successional stage on the primary productivity or respiration of the assemblages. In the other scenario of succession examined, the secondary succession, results confirmed the predicted positive effects of species richness and evenness in the performance of the assemblages at the latest successional stages.

Keywords: *Biodiversity-Ecosystem functioning, Coexistence mechanism, Successional dynamics, colonization-competition trade-off, macroalgae.*

4.1. Introduction

Aggregate ecosystem properties closely linked with global geochemical processes, like productivity, decomposition rates, nutrient cycling, etc., are being modified at increasing rate and scale as a consequence of the steadily global reduction of diversity (Loreau et al., 2001; Hooper et al., 2005). Over the last two decades, ecologists enthusiastically engaged in a joint research effort to appreciate the consequences of the global loss of diversity. The observed results of all this research effort are consistent with theoretical predictions: biodiversity has positive effects on ecosystems performance (Tilman et al., 2014). Hence, hundreds of experimental studies from terrestrial, aquatic, and marine ecosystems confirmed that high-diversity mixtures are approximately twice as productive as monocultures of the same species and that this difference increases through time (Tilman et al., 2014).

When considering horizontal diversity, i.e. the diversity of species within trophic levels or functional groups (Loreau & Kinne, 2010), theoretical work and experimental studies have often found saturating positive relationship between diversity and ecosystem functioning is the most common described pattern (Hooper et al., 2005; Tilman et al., 2014). Two different types of non-exclusive mechanisms could explain the relationship between average primary productivity and initial species diversity that was observed in biodiversity experiments. The first class of mechanisms involves positive complementarity effects, resulting from resource partitioning and/or facilitation (Hooper, 1998; Tilman, 1999). Complementarity resource partitioning results from differential resources used by different species as a result of evolutionary niche separation (Tilman et al.,1997). Facilitation, here used as synonymous with positive interactions, represents benefits provided by one species that can increase the effective niche of other species by habitat amelioration, enhanced recruitment or predation refuge; and consequently results in higher productivity (Bruno et al., 2003). The second type of mechanisms involves an effect of interspecific interactions known as the sampling effect, which is related with the higher chance of including the most productive species in randomly assembled mixtures of the higher diversity treatments (Huston, 1997, Aarssen et al., 2003).

Despite the mounting evidences of a positive relationship between biodiversity and ecosystem functioning found in experimental studies, monitoring studies in natural communities have found some conflicting results (Huston & McBride, 2002). These discrepancies have been attributed to the different nature of the community assembly processes involved in experimental and natural assemblages or to the successional stage of the communities (Thompson et al., 2005). Recently, mathematical models have demonstrated that some coexistence mechanisms (immigration processes, competition-colonization trade-off in successional transitions, disturbance regime) operating at both local and regional scales may drive different relationships to the described positive relationship (Loreau & Mouquet, 1999); (Cardinale et al., 2000); (Mouquet & Loreau, 2002). Thus mechanisms through which species coexist may determine the way species drive the functional role of diversity in communities. For example, high functional redundancy among species may prevent the existence of a positive effects between diversity and productivity (Loreau, 1998). However, most of these ideas have not been experimentally tested. This chapter aims to examine the functional consequences of diversity throughout one coexistence mechanisms extensively studied on intertidal assemblages: succession.

Together with disturbance, succession is recognized as one of the primary processes shaping landscape in ecosystems. Ecological succession consists of the sequence of changes in community structure that occur after a site has been disturbed (Connell & Slatyer,1977). Succession is being frequently seen as an orderly process of community development, quite directional and predictable. Odum (1969) highlighted that succession is mostly a community-controlled process that culminates in “stabilized ecosystems in which maximum biomass and symbiotic functions among organisms are maintained”. However, chance is also relevant in ecological succession, and Clements in the early nineteen century had already recognized the importance of colonization chance (Lawton,1987). Whether succession is largely a canalized or a contingent process, i.e. deterministic or stochastic, is still a large subject of debate (Berlow, 1997)
Studies on intertidal communities have played a key role on the comprehension of ecological succession (Berlow, 1997). In fact the three mechanisms identified as the most relevant driving the successional processes were first describe on intertidal assemblages (Connell & Slatyer,1977). Three were the succession mechanism described by these authors: i) facilitation, where early stages are necessary for subsequent development; ii) inhibition, where early stages preclude the colonization of

later stages and it is considered as the most common mechanism operating in natural systems iii) tolerance, where succession progresses due to the life history characteristics of the organisms, rather than positive or negative interactions (Connell & Slatyer, 1977).

Nowadays, new theoretical frameworks on the coexistence mechanisms operating throughtout succession have been suggested. Pacala & Rees (1998) highlited two alternative mechanisms promoting successional diversity. The first is the competition-colonization in which early successional species are able to persist because they colonize disturbed habitats before the arrival of late successional dominant competitors (Tilman,1994). The second is the successional niche hypothesis in which, even with unlimited colonization by late successional dominants, early colonizers may temporarily to outperform late successional because they specialize on the resource-rich conditions typical of recently disturbed sites. In the case of primary succession, i.e. that one occurring after the creation of new space, competition-colonization trade-offs among species are considered to be the key mechanisms driving succession (Tilman, 1994). In the case of secondary succession which occurs after a disturbance that partially removes existing biota, successional niche has been advocated as the main coexistence mechanisms. Both mechanisms of species coexistence were suggested as having a different effect of biodiversity-ecosystem relationship that other forms of coexistence like niche differentiation. To date, studies focused on successional diversity and ecosystem functioning are mostly theoretical approaches, with findings depending on model assumptions (Cardinale et al., 2004; Kinzig & Pacala, 2001).

In this chapter, we set up an experiment to examine how the relationship between diversity and ecosystem functioning changes through time during the successional transition using algal assemblages. There are obvious benefits of exploring biodiversity-ecosystem functioning questions using this type of assemblages, including the ease to manipulate the biota, the short-time scales, the existence of methodologies for measuring ecosystem processes and the general good knowledge of the system.

Based on previous theoretical models we hypothesize:

1) In the scenario of primary succession where the colonization-competition trade-off dominate the community dynamics, species richness has not relationship with primary productivity. In this case highly productive species may dominate assemblages particularly during the early stage of succession and create low diversity but highly productive assemblages.

2) In the scenario of secondary succession, where interactions among species are the major drivers of the succession dynamics (i.e. successional niche processes *sensu* (Kinzig & Pacala,2001) we expect that biodiversity will positively affect primary productivity particularly at the late stage of succession.

To test both hypotheses, we setup an experiment using macroalgal assemblages where we created two successional scenarios, i.e. primary and secondary succession and measured the relationships between primary productivity and two biodiversity proxies (species richness and evenness) at two different stages of the succession (early and late succession).

4.2. Materials and Methods

4.2.1 Study area

The experiment was conducted between October 2011 and May 2013 at Praia Norte (Viana do Castelo, Portugal, 41°41′27″N, 8°50′57″W). This shore, like all the Portuguese coastline, is influenced by a semidiurnal tidal regime. Praia Norte is a granitic and slate rocky shore exposed to prevailing northwest oceanic swells that are particularly intense during the winter (Bertocci et al., 2010).

We used artificial substrates created from natural stone to examine the functional consequences of the successional changes on diversity. Thus, we constructed synthetic assemblages which consisted of 12x 17x1cm PVC plates with 16 pieces of rock surrounded by 1 cm PVC pieces for support and protection (Figure4.1).The pieces of rock were created from stones collected from Viana do Castelo and cut in cubes of 2x2x2 cm which were held in position using underwater setting cement and stainless steel screws. Each plate provided around 80 cm^2 as available surface for the seaweed assemblage.

Figure 4.1.Macroalgal assemblage plate used in the study.

We aimed to examine the process in two different scenarios: primary succession and secondary succession. Hence (Arenas et al., 2009) we built two types of plates: i) plates with clean rock and ii) plates with a perturbed seaweed assemblage. In the first case, rock cubes were scraped and then submerged in a 10 % HCL bath for 48 hours. In the second case seaweeds were scraped vigorously to remove all erect fronds like it happens in natural disturbances due the impacts of waves during severe storms (Underwood,1998) Both types of plates were constructed in the same way with the same set of stones, but they were randomly assigned to the two types of successional scenario and subsequently underwent the different treatments.
A total of 40 plates were built and placed haphazardly in a set of rockpools. From the original plates finally some of them were lost or broken; hence only 17 were used for primary succession and 20 for secondary succession.

Plates were deployed in the intertidal area of Praia Norte, Viana do Castelo. They were screwed to the bottom of suitable large rockpools haphazardly chosen (at an average depth of approx. 30 cm) and the development of new algal assemblages and their performance was monitored after three, six and 12 months and one final additional sampling was carried out after 21 months. Sampling after 3 & 6 months were considered as early succession stages and sampling after 12 & 21 months were considered as advanced successional stages. Sampling in months 3 & 12 occurred in late autumn-winter period. Sampling at 6 and 21 months occurred in spring early summer when richness and biomass reach the highest values in this shores.
At each monitoring date, plates with the assemblages were taken from the shore and transported in boxes back to the laboratory. To reduce any damage due to desiccation during the transport, the assemblages were kept wet using clothes damped with seawater. Once in the laboratory, assemblages were placed in a 300 L tank which seawater connected to a water pump to recirculate the seawater (filter water) at 15 C° and with a permanent air supply.
Before starting the incubations, the PVC sides of the plates were cleaned using scrapers to remove all the fouling organisms attached outside the stone mosaic area (approx. 80 cm^2) (Figure 4.2).

Figure4.2.Cleaning process of the surrounded plate area.

The incubations were performed at the Laboratory of Coastal Biodiversity, at Ciimar in Porto (Portugal). Incubations were carried out in a specifically constructed incubation chamber with temperature and light control facilities. In each monitoring date the incubations were carried out in less than three days and afterwards plates were returned to the shore using the same procedure described above.

4.2.2 Structural and functional measurements

The structural measures include species richness and cover estimations for all the macroalgal species present at the assemblages at each sampling time. Functional measures consist of estimations of primary productivity and respiration rates through oxygen fluxes at different light irradiances (Arenas et al., 2009). Incubations were carried out under controlled temperature (Figure 4.3). Light levels were created using several sets of fluorescent tubes (Osram® Biolux) which were switch on sequentially to create 6 irradiance levels, 0 (dark period), 40 (L1), 115 (L2), 188 (L3), 253 (L4) , 319 (L5) and 450 µmol photons m^{-2} s^{-1} (L6 full light). Incubations ran for approximately 2 hours, with around 20 minutes for each light period except in the case of the dark period to estimate respiration, which usually lasted 30 minutes. These times ensured the accommodation of the assemblages to each irradiance level and linearity in the response of oxygen fluxes.

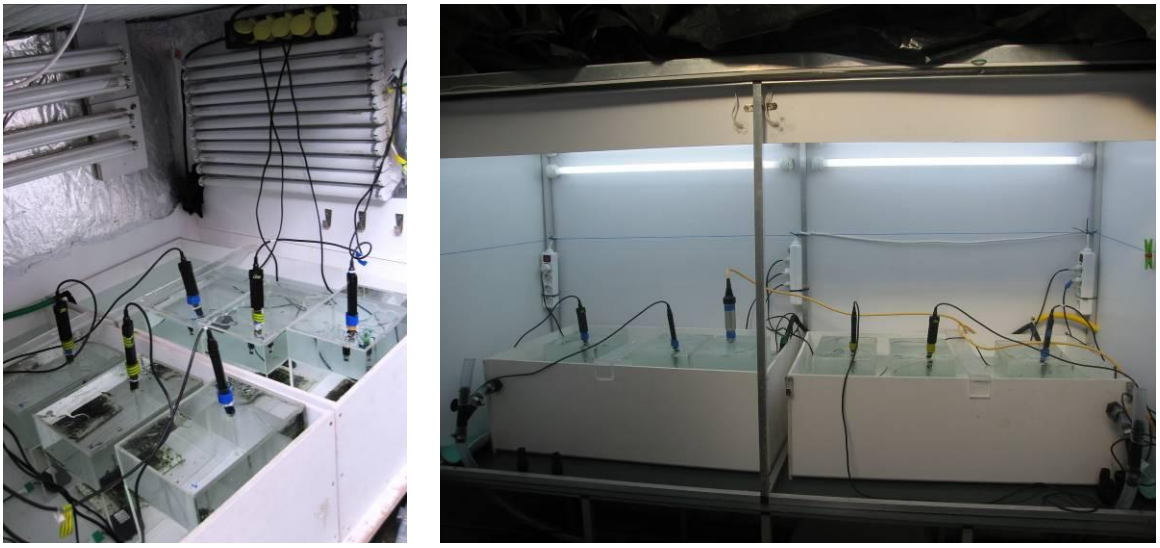


Figure 4.3.Incubation procedure with the large CT chamber, temperature controlled seawater bath and the small incubation chambers where oxygen fluxes were measured.

The incubation facility included two large seawater baths at a constant temperature of 15 ºC controlled using a seawater chiller (Teco® 15T). We use carry out six incubations simultaneously using acrylic incubation chambers which consisted in 12.5 litters sealed acrylic chambers equipped with a small submersible pump with a diffuser to create water movement without excessive turbulence. Incubations were carried out always using 5 µm pre-filtered seawater.

Productivity-irradiance (P-I) curves were estimated by measuring oxygen fluxes inside the chamber. Oxygen concentration was recorded every thirty second using a dissolved oxygen probe with luminescent technology (HACH) connected to a data logger.

Four ecosystem functioning surrogates were determined per assemblage: (Arenas et al., 2009) (1) maximum net primary productivity (max NPP), the maximum productivity (i.e. maximum slope for the oxygen concentration versus time relationship) recorded at any light intensity (mg O_2 h^{-1}); (2) Gross primary productivity (GPP);(3) assemblage respiration, the oxygen consumption during the dark period of the incubation (mg O_2 h^{-1}); and (4) photosynthetic efficiency at low irradiance (α), estimated using ordinary least squares (OLS) regressions for the light-limited portion of the curve (mg O_2 µmol $m^{-2}s^{-1}$).

Immediately after the last incubations in May 2013 (21months), macroalgae were scraped from plates sorted by species and dried at 60ºC for 48h to estimate dry biomass (g dry weight, DW) in our plates at the end of the experimental period.(Figure 4.4 a), b)).

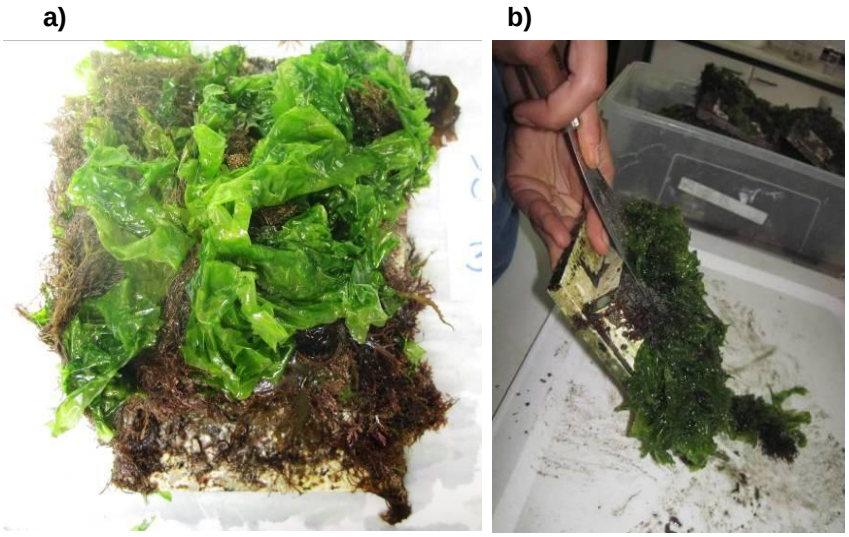


Figure4.4. a) Plate after incubation and b) Biomass separation procedure.

4.3. Statistical analyses

Compositional trajectories for the two successional scenarios were examined by comparing the centroids describing the average assemblages for each succession treatment over the course of the study. To obtain the centroids, data from all the assemblages of each succession treatment and sampling time were first averaged and a dissimilarity matrix based on the Bray-Curtis index was generated among the full set of 148 assemblages (17+20 assemblages × 4 sampling dates). Because of the nature of the Bray-Curtis index, centroids were calculated using principal coordinates (PC) from the from the Bray-Curtis dissimilarity matrix (Anderson 2001). Centroids were then obtained as arithmetic averages of the principal coordinates over the 4 sampling dates (McArdle & Anderson, 2001) using the software Primer+Permanova v6 (Clarke & Gorley,2006). Additionally, simper analyses were used to define which species contributed the most to the differences among the two diversity scenarios.

Univariate structural descriptors like species richness and evenness were examined using linear mixed models (Bolker et al., 2009). We included two fixed factors and two random terms. Fixed factors were: succession type (primary and secondary succession), succession stage (also with two levels: early and late). As random terms we included month (nested in succession stage) and assemblage identity. These models including simultaneously stage, succession type and the structural continuous predictor's richness & evenness could be over parametrized and yield complex third level interactions. Thus we decide to run separate analyses on the relationships between the structure and the performance of the assemblages for primary and secondary successional types. All the analyses were carried out using R software and the package lme4 (Bates et al., 2014). We first identified the structure of the random effects. Maximal full models were then fitted using all the fixed terms predictors and their interactions. Next, minimum adequate models (MAMs) were identified using a successive backward selection procedure. MAMs were selected using Likelihood Ratio Tests and as the ones that minimized Akaike Information Criterion (AIC). To examine models assumptions we plotted deviance residuals against fitted values and performed qqplots.

4.4. Results

4.4.1. Assemblages changes through the two successional scenarios

Three months after the deployment of the plates, assemblages from primary and secondary succession plates looked very different. In the primary succession treatments the opportunistic *Ulva* spp. was the most abundant species (average cover 34.6 % versus 13.2 %) in secondary succession assemblages (Table 4.1). Conversely, perennial species like *Corallina spp*, *Lithophyllum incrustans*, *Chondrus crispus* and *Sargassum muticum* partially recovered from the scrapping and were already profuse in secondary succession plates (18.6, 6.5 and 5.4 % of average cover in those assemblages respectively). Simper analyses demonstrated that these species were among those contributing to more than 80 % of the differences between both successional types. In April, six months into the experiment, *Ulva spp* reduced its abundance on primary succession plates (9.2 %) while some perennial species were already present on those plates (e.g., *Lithophyllum incrustans*: 7.2 %; *Corallina* spp: 1 %). Changes in the secondary succession assemblages were less intense and at this time differences between both succession treatments were mostly due to differences in the cover of *Corallina spp* (on average 25.4 % in secondary succession ones).

During the two last sampling dates (i.e., 12 and 21 months after the start of the experiment), *Ulva spp* and *Corallina spp* remained as the most abundant species in cover in primary and secondary succession assemblages respectively and were among those explaining most of the differences between treatments (Table 4.2).

For all the dates examined, ANOSIM analyses demonstrated that primary and secondary succession assemblages were significantly different (ANOSIM, $p<0.001$ for all cases). However the MDS plots for the PC centroids for both succession scenarios clearly show how the assemblages from primary and secondary succession started very far after three months of succession but ended having similar but parallel trajectories (Figure 4.5).

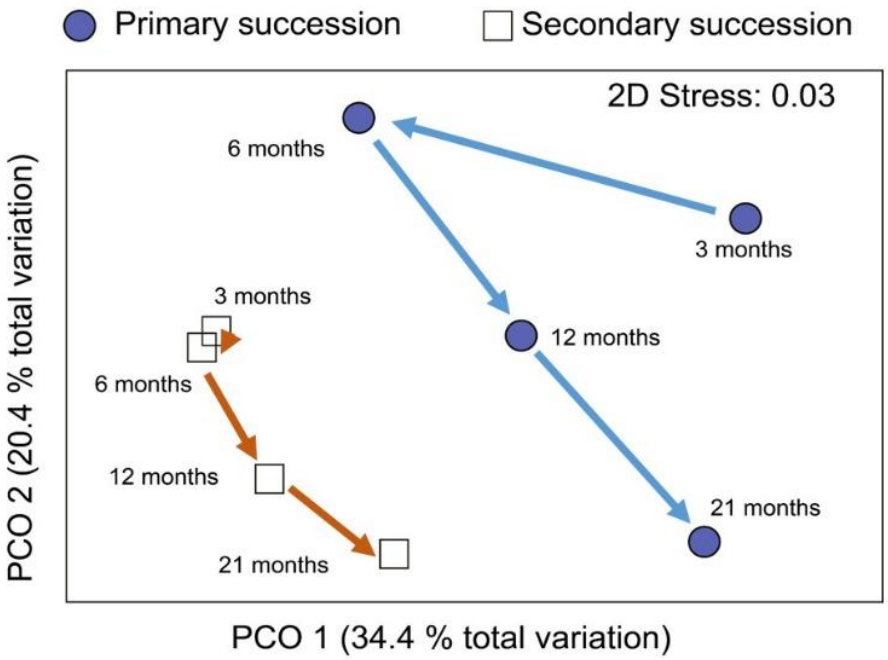


Figure 4.5. Non-metric multidimensional scaling ordination (nMDS) on the basis of Bray-Curtis dissimilarity measure for the centroids of the two successional scenarios and sampling data

Regarding the species richness and evenness descriptors, species richness seemed to increase gradually with time in primary succession treatments while in the case of secondary succession assemblages had no apparent trend after the initial drop of richness from the third to the sixth month. Similar pattern was found for evenness with a quick increase after three months of succession and later remained stable in primary succession plots. No trends were found for the secondary succession treatments.

Linear mixed models (LMM) to examine those differences on species richness and evenness between early and late succession stages on both primary and secondary succession treatments found a significant interaction Succession type X Succession stage. Tukey HSD post hoc test showed that differences on species richness and evenness from primary and secondary assemblages were just significant for the first sampling date (three months, Tukey HSD t test, $p<0.05$) and no differences among for the rest of the dates (Tukey HSD t test, $p>0.05$ in both cases).

Table4.1.Output of the simper analysis with the species which contributed most to the dissimilarities between the two scenarios of succession examined in the study for the two first sampling dates. Simper analyses performed after fourth square root transformation of cover data.

3 Months	**% Cover**		
Average dissimilarity among succession treatments: 63.7	Primary Succession	Secondary Succession	Cum % Dissimilarity
Corallina spp.	0.12	18.6	22.33
Lithophyllum incrustans.	0.4	6.7	37.82
Chondrus crispus.	0	5.4	47.09
Chondracanthus teedei.	0.4	1.1	54.86
Sargassum muticum.	0.1	1.8	62.5
Ulva spp.	34.6	13.2	69.9
Ceramium spp.	1.8	0.3	76.05
Osmundea pinnatifida.	2.2	0.1	81.9

6 Months	**% Cover**		
Average dissimilarity among succession treatments: 44.6	Primary Succession	Secondary Succession	Cum % Dissimilarity
Corallina spp.	1	25.4	31.9
Ceramium spp.	0.2	1.7	42.9
Chondracanthus teedei.	0.9	1.3	53.8
Ulva spp.	9.2	6.8	63.4
Lithophyllum incrustans.	7.2	12.1	71.4
Chondrus crispus.	0.1	1.3	79.3
Sargassum muticum.	0	3.3	85.6

Table4. 2. Output of the simper analysis with the species which contributed most to the dissimilarities between the two scenarios of succession examined in the study for the two last sampling dates. Simper analyses performed after fourth square root transformation of cover data.

12 Months	**% Cover**		
Average dissimilarity among succession treatments: 52.3	Primary Succession	Secondary Succession	Cum % Dissimilarity
Corallina spp.	2.35	37.5	24.9
Gigartina pistillata.	1.2	6.4	36.2
Lithophyllum incrustans.	4.4	5	47.4
Gelidium sp.	3.2	1.9	57.3
Cladophora sp.	1.5	0.6	63.6
Sabellaria sp.	0.5	1.3	69.7
Ulva spp.	22.5	13.6	74.7

21 Months	**% Cover**		
Average dissimilarity among succession treatments: 52.2	Primary Succession	Secondary Succession	Cum % Dissimilarity
Corallina spp.	6.8	29.5	14.6
Osmundea pinnatifida.	10.3	2.3	25.6
Ceramium spp.	2.9	6.6	35.45
Gelidium sp.	7.2	7.1	44.76
Lithophyllum incrustans.	1.5	4.6	53.9
Gastroclonium ovatum.	2.2	8.1	62.6
Grateloupia filicina.	5.11	0.2	70.8
Chondracanthus teedei.	1.3	3.8	77.8
Ulva spp.	32.8	17.7	82.3

4.4.2 Biodiversity-Productivity relationships through the successional process

4.4.2.1 Primary succession

Despite the apparent overall positive relationship between the structural predictors (i.e. species richness and evenness) and functional response variables for all the assemblages and dates in the primary succession treatment (figure 4.6), our models did not find relevant effects neither for any of our structural predictors or the successional stage (early and advanced succession) in the assemblages' gross primary productivity (GPP), net primary productivity (NPP) or respiration (RESP). All those models found large variability in the random term of dates within stages (see figure 4.7).

Only the photosynthetic efficiency at limited light conditions we found relevant factors that change the model significantly (ANOVA, $p<0.05$) when removed although they were not always significant through the process of model reduction. In particular, our model suggests an interaction between the continuous predictor evenness and successional stage (Table 4.3). Assemblages increased in efficiency in the succession process, although the relevant interaction between those two descriptors indicated first, that the effect of evenness on alpha reversed from negative in the first part of succession to nonnegative in the late-succession stage.

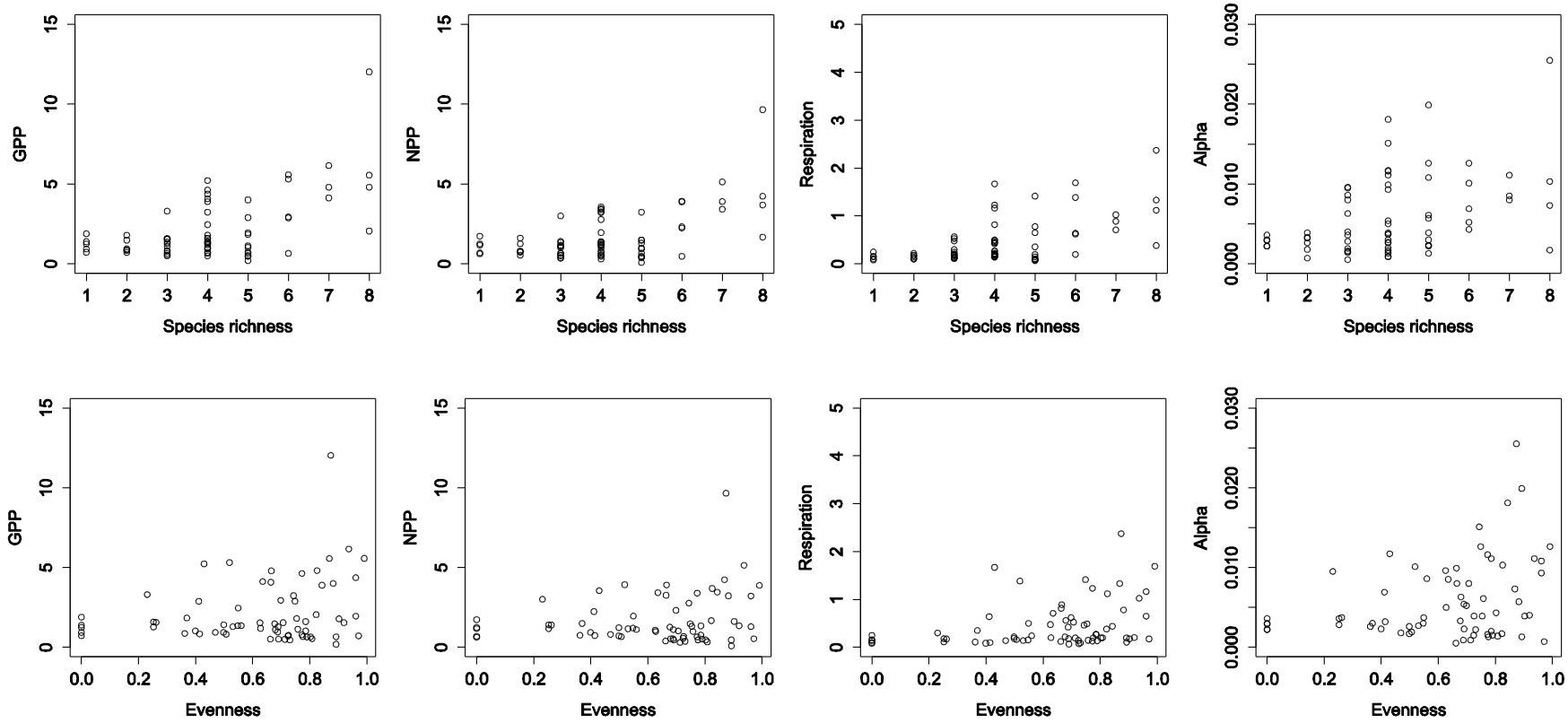
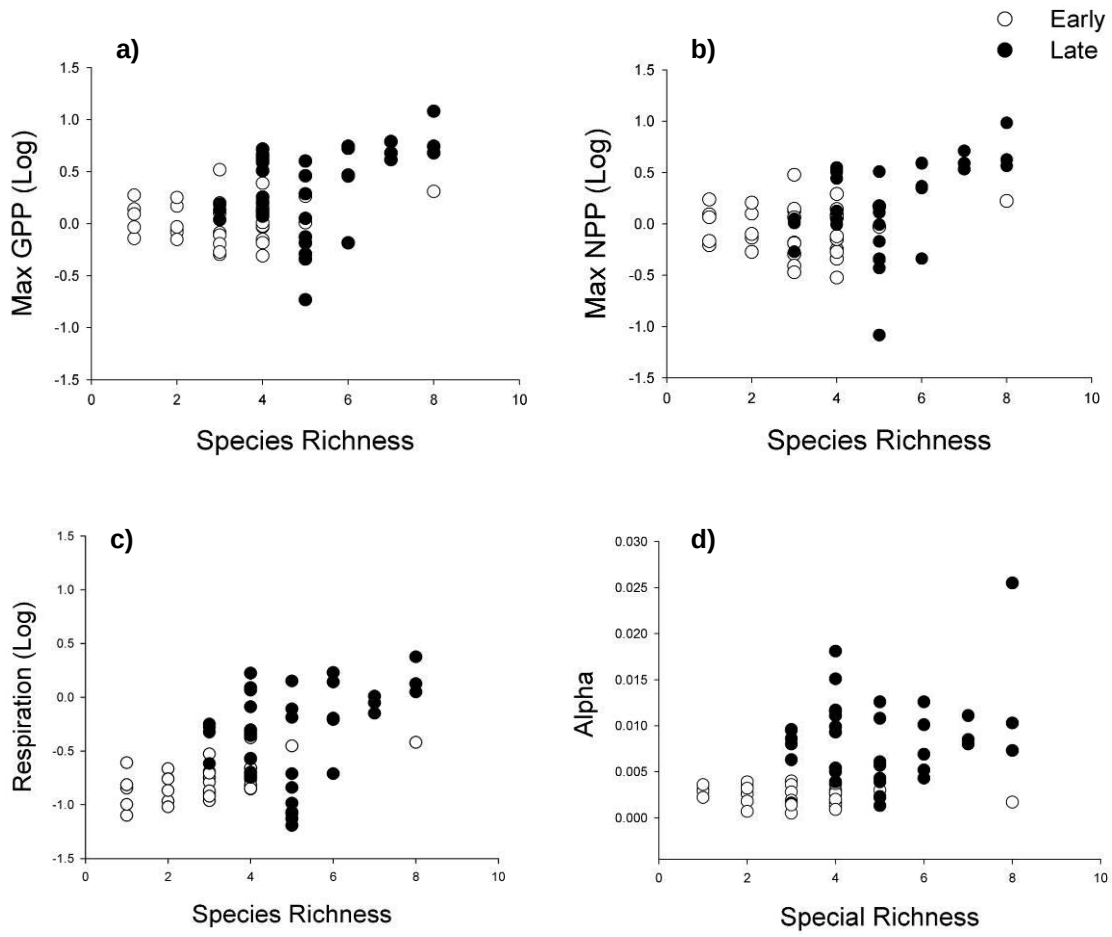


Figure4.6. Overall relationship between structural predictors (i.e. species richness and evenness) and functional response variables for all the assemblages and dates in the primary succession treatment. Each does correspond to one assemblage. GGP, NPP and Respiration in mg O_2 assemblage^{-1} h^{-1}. Alpha in mg O_2 µEm^{-2}s^{-1}.

In fact, alpha increased during the primary succession process and the average triplicate from the early to the late succession stage (0.0032±0.0004 (SE) in the early succession to 0.0109±0.001 (SE) (mgO_2 µE $m^{-2}s^{-1}$) in the late-succession, n=36 in both conditions).

Table4. 3.Summary of the minimum adequate linear mixed model for the functional variable alpha during the primary succession. Only fixed terms are included. Predictors were not significant.

Alpha – Primary Succession			
Fixed terms	**Estimation**	**SE**	**t value**
(intercept)	-5.98	0.33	-17.82
Evenness	-0.27	0.34	-0.77
Succession stage	0.84	0.65	1.29
Evenness x Stage	0.63	0.73	0.85



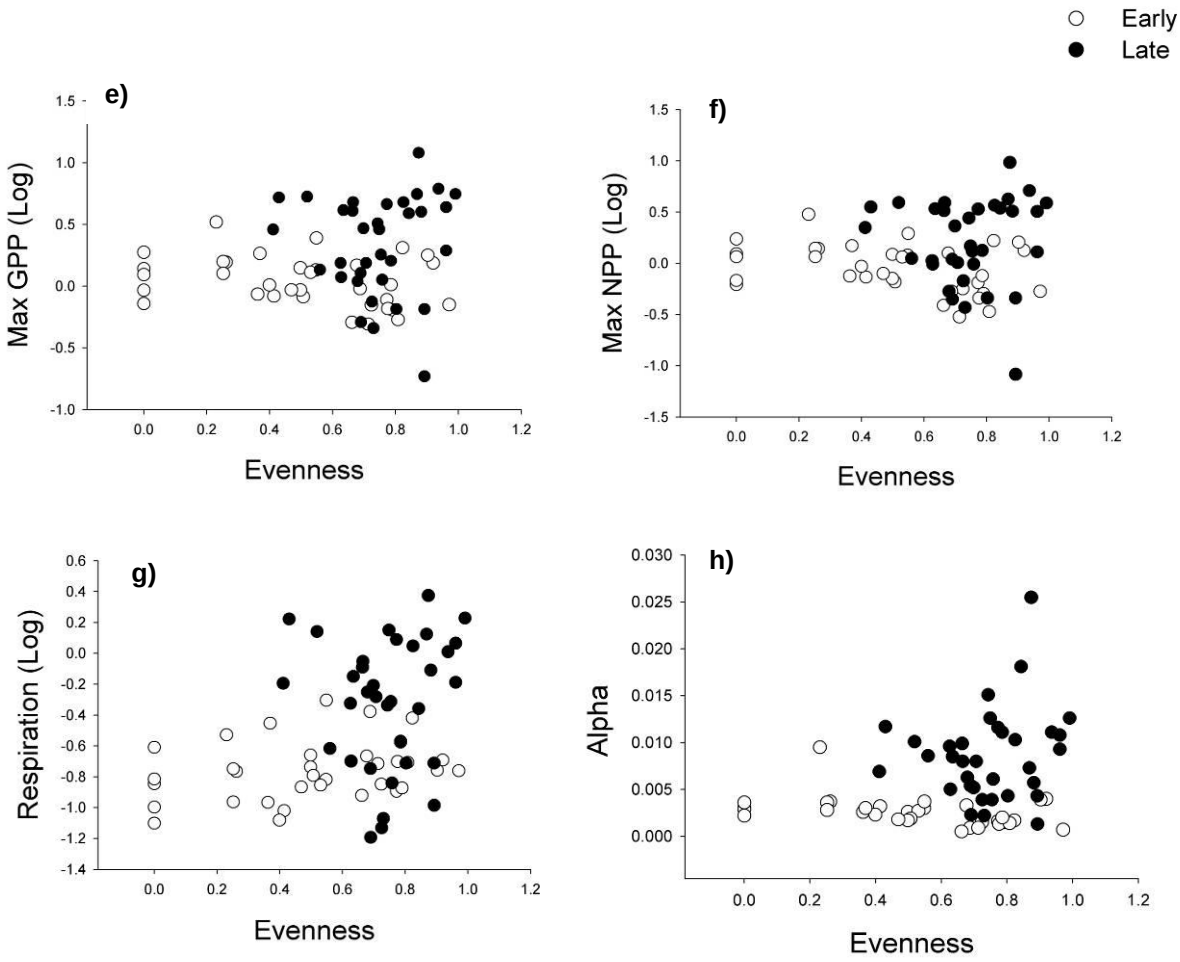


Figure 4 .7 Relationship between structural predictors (i.e. species richness and evenness) and functional response variables for all the assemblages early and late in the primary succession treatment. a) gross primary productivity (Max GPP) and species richness, b) maximum net primary productivity (max NPP) and species richness , c) respiration and species richness, d) photosynthetic efficiency (alpha) and species richness, e) gross primary productivity (Max GPP) and evenness, f) maximum net primary productivity (max NPP) and evenness, g) respiration and evenness and h) photosynthetic efficiency (alpha) and evenness.

4.4.2.2. Secondary succession

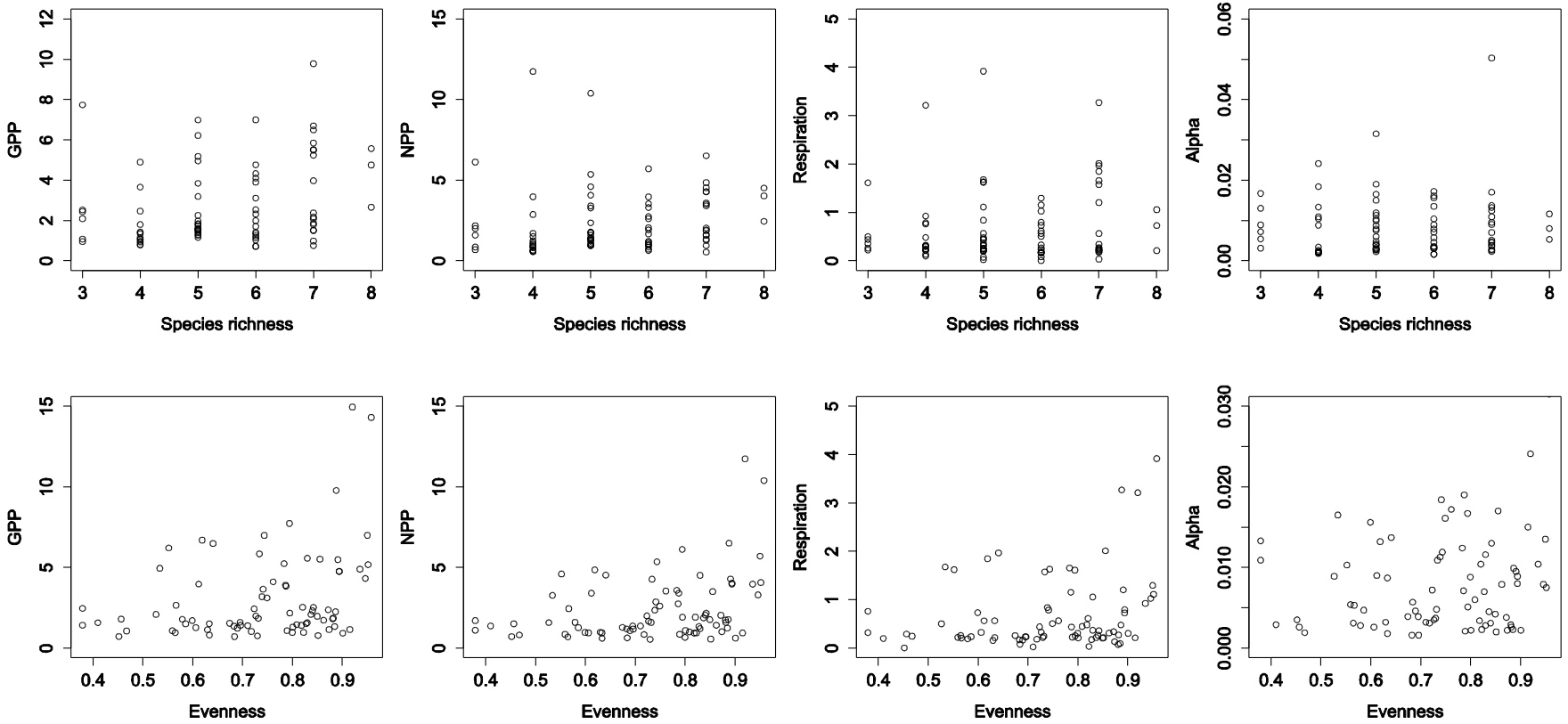


Figure 4.8. Overall relationship between structural predictors (i.e. species richness and evenness) and functional response variables for all the assemblages and dates in the secondary succession treatment. Each does correspond to one assemblage. GGP, NPP and Respiration in mg O_2 assemblage $h^{-1.}$ Alpha in mgO_2 µE $m^{-2}s^{-1}$.

Figure 4.8 shows the functional secondary succession did found more relevant and significant predictors. As in the case of the primary succession treatment, looking into the whole dataset, both diversity and evenness seems to have a positive effect on the functioning proxies measured. When analyzing the data including all its structure, i.e., incorporating random terms and considering interactions among predictors we found some relevant predictors. In the case of GPP & NPP, species richness, evenness and succession stage had a positive effect on those functional parameters but the negative coefficient of interaction Species richness x stage suggest that the slope in the relationship between richness and primary productivity decreased in the late-successional stage (Table 4.4a,b). In the case of respiration, only successional stage was a relevant predictor with a positive effect, i.e. respiration rates were higher in the late-successional stage (Table 4.4c). Finally, the efficiency variable, alpha, was positively influenced by assemblages richness and evenness and also increased it value in the late-successional stage (Table 4.4d), (Figure 4.9).

Table4.4.Summary of the minimum adequate linear mixed model for the functional proxies a) GGP, b) NPP, c) Respiration and d) Alpha during the secondary succession. Only fixed terms are included.

a) GPP – Secondary Succession			
Fixed terms	**Estimation**	**SE**	**t value**
(intercept)	-1.24	0.54	-2.28
Species richness	0.21	0.06	3.48
Evenness	0.67	0.34	1.96
Succession stage	2.06	0.67	3.06
Species richness x stage	-0.22	0.08	-2.77

b) NPP – Secondary Succession			
Fixed terms	**Estimation**	**SE**	**t value**
(intercept)	-1.64	0.54	-3.01
Species richness	0.23	0.06	3.84
Evenness	0.78	0.35	2.21
Succession stage	2.20	0.67	3.29
Species richness x stage	-0.27	0.08	-3.25

c) Respiration – Secondary Succession			
Fixed terms	**Estimation**	**SE**	**t value**
(intercept)	-1.66	0.48	-3.47
Succession stage	1.43	0.67	2.12

d) Alpha – Secondary Succession			
Fixed terms	**Estimation**	**SE**	**t value**
(intercept)	-6.50	0.34	-19.10
Species richness	0.09	0.04	2.10
Evenness	0.44	0.38	1.16
Succession stage	1.23	0.11	11.34

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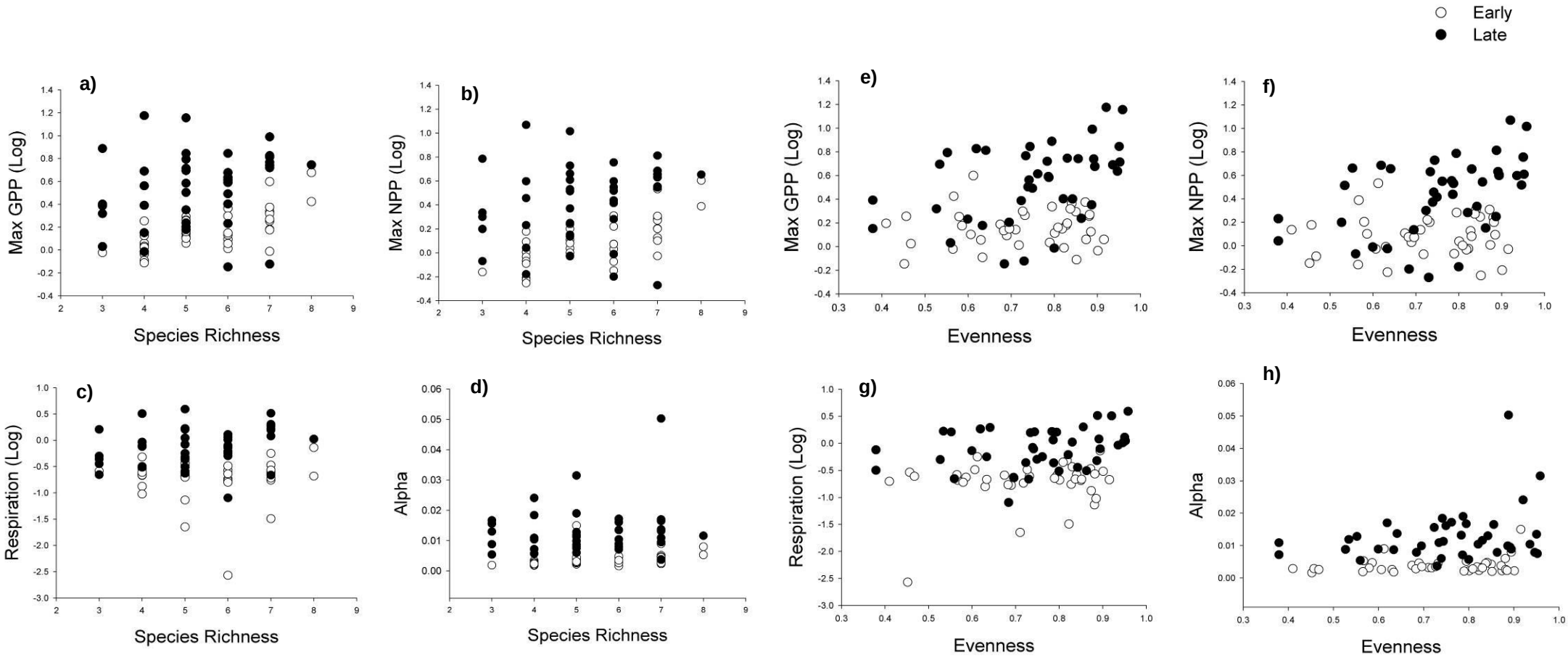

Figure 4.9. Relationship between structural predictors (i.e. species richness and evenness) and functional response variables for all the assemblages early and late in the secondary succession treatment. a) gross primary productivity (Max GPP) and species richness, b) maximum net primary productivity (max NPP) and species richness , c) respiration and species richness, d) photosynthetic efficiency (alpha) and species richness, e) gross primary productivity (Max GPP) and evenness, f) maximum net primary productivity (max NPP) and evenness,g) respiration and evenness and h) photosynthetic efficiency (alpha) and evenness.

4.5. Discussion

This is among the first studies which explicitly tested the functional effects of changes in species diversity through succession in marine systems. To perform this study, we used intertidal macroalgal assemblages as model assemblages. These assemblages are very convenient for this type of experimental approach because succession is a relatively quick process compared to their counterpart terrestrial systems (Sousa, 1979), (Viejo et al., 2008). Nevertheless as differences in the multivariate analyses showed, twenty one months after the total removal of biomass in the primary succession scenario, assemblages were still different from secondary assemblages and early colonizers like Ulva were the most abundant species on those plates.

The study examined only some primary productivity related parameters, however we feel that the precision of our measure provide confidence to the results. This confidence is enhanced by the correspondence of our results with some of the predictions available from theoretical models (Kinzig & Pacala, 2001).

At the spatial scale used in this study (i.e. 80 cm^2) and considering separately early and late successional stages, ecosystem functioning proxies examined here did not performed always as expected from other experiments with macroalgal assemblages (Bruno et al., 2005; Bruno et al., 2006; Boyer et al., 2009) and observational studies (Arenas et al., 2009), where higher diversity assemblages always showed on average higher standing biomass or primary productivity.

During the succession, species-specific productivity decreases with time and biomass accumulation increases (Littler & Littler, 1980). In the assemblages where the primary succession occurred, there were no effects of diversity, evenness or successional stage on the primary productivity or respiration of the assemblages. In the primary succession, the prevalence during the whole experiment of early colonizers with high productivity like *Ulva* spp. drove clearly all the diversity-productivity relationships. Early colonizers opportunistic species were able to reduce the subsequent colonization of late-colonizers species, i.e. the inhibition mechanism *sensu* Connell & Slatyer (1977), decoupling the relationship between diversity and productivity. Hence at early successional stage *Ulva spp.* dominated assemblages had low diversity but relative high productivity. In this condition *Ulva spp.* cover was positively related with primary productivity but diversity was uncoupled with productivity. Late in the primary succession those plots dominated by *Ulva spp.* had lower diversity and less

productivity, thus diversity should be positively related to productivity, but this positive effect was only found in spring after 21 months, in the other late succession sampling data, performed in winter after 12 months of succession the trend was negative, probably due to existence of many small individual of perennial species which were partially inhibited by the *Ulva spp.* cover, added species number but not much biomass and thus productivity. Also due to winter dormancy period of some species like *Sargassum muticum* or *Cystoseira* could explain partially these results.

This lack of effects of primary succession on the relationship between diversity and primary productivity are in agreement with other succession experiments carried out on macroalgal assemblages (Nöel et al., 2009). For instance, (Martins et al., 2007) found no difference in community productivity between early-successional and undisturbed control pools, probably because the higher biomass on the control pools ones compensated the higher productivity of the opportunistic species abundant in perturbed areas.

Unlike the other primary productivity proxies, the photosynthetic efficiency of the assemblages under low light conditions (alpha) did change across succession stages and evenness levels. Assemblage increased in efficiency in the succession process, although this increases was also shaped by the distribution of biomass within the assemblages. Assemblages' efficiency, as it was measured here, was able to capture some of the subtle changes that were occurring through the successional process, where the species slowly adjusted each other and the assemblages became more and more efficient using resources, although these subtle did not fully modified the ultimate proxies like GPP, NPP and respiration.

In general results from assemblages productivity under the primary successional scenario agreed with the predictions made by (Kinzig & Pacala, 2001) which concluded that assemblages controlled by colonization-competition dynamics effects of diversity on functioning could be either positive, neutral or negative, depending on the species present and the performance trade-offs that may accompany colonization-competition trade-offs.

In the other scenario of succession examined, the secondary succession, results confirmed the predicted positive effects of species richness and evenness in the performance of the assemblages. However the sign of the interaction species richness and succession stage suggested that the effect of diversity was more intense during the early succession period. Assemblage's efficiency also increased with species richness, evenness and time, confirming that species inside the plates were progressively fitting each other.

Our findings also agree with the predictions made by the models of (Kinzig & Pacala, 2001) where the late successional species dominance is not delayed by the persistence of early-successional. In our assemblages, late successional species quickly dominated the assemblages soon after the perturbation suggesting that the proposed mechanisms may be relevant on natural systems.

All those patterns described and mechanisms suggested so far only refer to the local interactions processes occurring at the small assemblage scale, at each individual plate.

However if we look to the whole dataset at one, a positive diversity trend in most of the examined functional proxies is clearly observable. This trend is probable similar to the trends that we could measure at landscape level studies, where multiple succession stages and environmental conditions simultaneously shape the relationship between diversity and functioning.

In her book chapter Nöel and colleagues (2009) stated that none to integrate successional concepts with current theories, we need to understand both (1) the extent to which diversity contributes to differences in ecosystem functioning during succession and (2) whether the effect of diversity depends on the stage of succession.

We think that this chapter is a good approximation to both requests and brings some light on how diversity-primary productivity interrelates through the process of succession.

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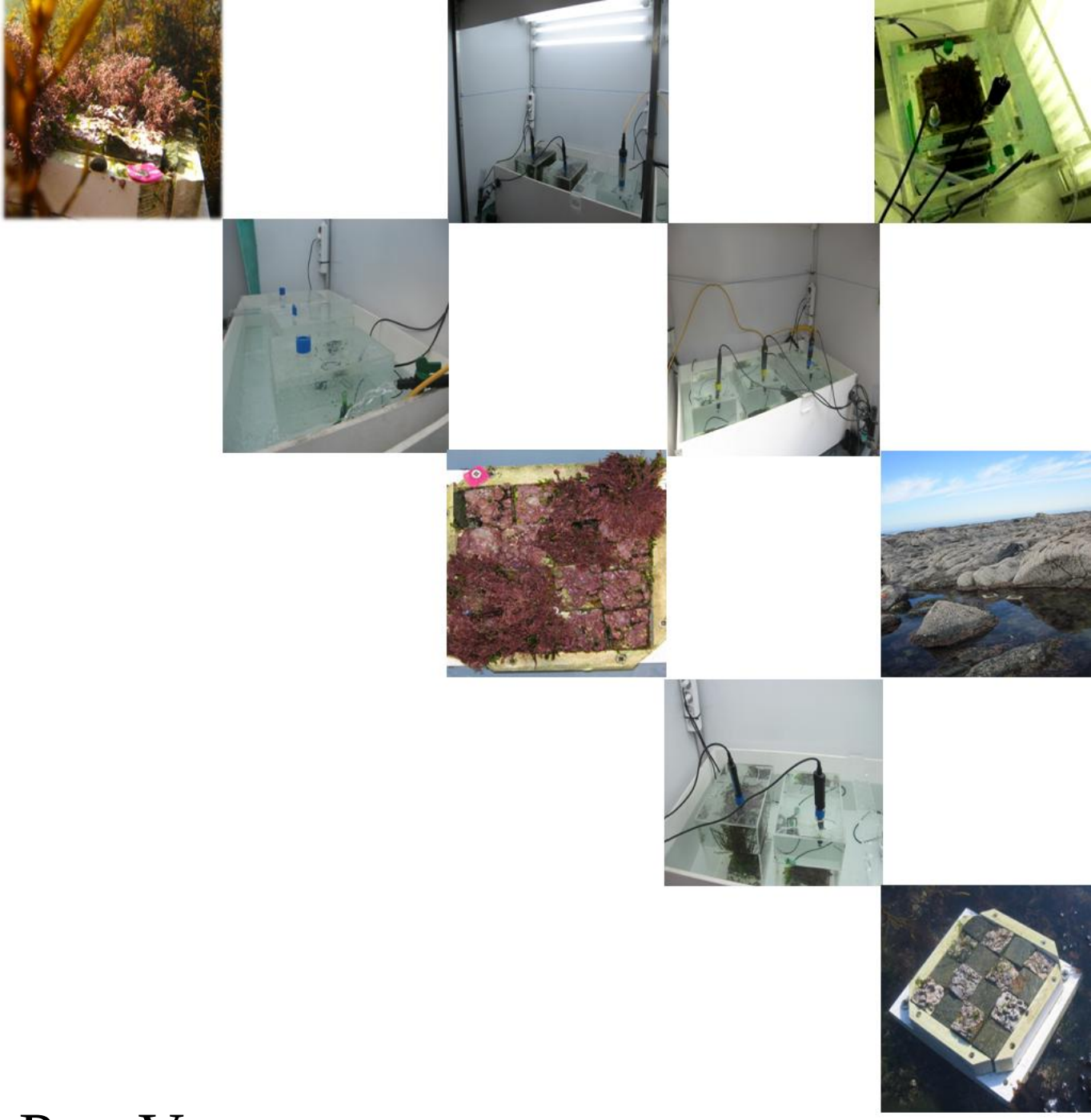

Part V

Chapter 5

Seeweed in the space: the effects of patchiness on the functioning of macroalgal assemblages

Abstract

Ecosystem properties like productivity are being modified as a consequence of the global reduction of diversity. Over the past decade, theoretical and experimental studies have generally found a positive relationship between diversity and ecosystem functioning due to a complementary resource use among species. To date, most of the experimental studies have been based on mesocosms units where the spatial distribution of the species is typically random. However in natural communities, spatial distribution of plants and animals is typically non-random. These spatial patterns may be relevant to ecosystem functioning, namely for sessile species because local spatial arrangement determines the intensity of interactions among individuals. However, community's spatial aggregation has received little attention in the context of diversity-functioning research.We used intertidal macroalgal communities to build 72 synthetic assemblages. We created communities with 2 levels of functional diversity (1 and 2 functional groups) and 3 different spatial aggregation patterns: assemblages with 2 patches, 4 patches and 8 patches while controlling for the overall biomass. We measured primary productivity of the assemblages after four months in the shore. Our finding confirm clear and consistent effects of patchiness on systems performance with higher productivity levels on those plots with higher patchiness. Our results highlight the importance of incorporating spatial structure and heterogeneity on ecosystem functioning research.

Keywords: *Patchiness, Spatial aggregation, Productivity, Ecosystem Functioning*

5.1. Introduction

More than two decades after the onset of the research on biodiversity-ecosystem functioning relationships, ecologists are now fully aware that most of ecosystems processes in natural systems are largely shaped by their biodiversity (Tilman et al., 2014). Biodiversity, previously identify with number of species, is in this context a much wider concept and includes species trait identities and traits diversity, relative abundances of species and spatial arrangement (Hooper et al., 2005). There is also a consensus that ecosystem properties which are closely linked with global geochemical processes, like productivity, decomposition rates and nutrient cycling, are being modified at increasing rate and scale as a consequence of the steady global reduction of diversity, causing also economic impacts affecting ecosystem products and services to humankind (Cardinale et al., 2012; Hooper et al., 2012).The mounting evidence gathered through dozens of theoretical and experimental studies have helped greatly to articulate a set of hypotheses on the functional consequences of biodiversity which has been named as the new biodiversity-ecosystem paradigm (Naeem,2002; Gamfeldt & Hillebrand,2008). Essentially, research have found that effects of biodiversity on ecosystems are real and large, are often caused by complementarity and occur all type of systems, i.e. terrestrial, freshwater, and marine ecosystems (Tilman et al., 2014).

However, research on biodiversity-ecosystem functioning like any other new growing field of knowledge has raised many new questions, mostly related to how relevant is this research to natural systems, or in other words it is uncertain to what extent results can be extrapolated to natural systems. Concerns about the generality of results include how to scale up results from small scale experiments to the wider spatial and temporal scale of real ecological systems (Cardinale et al., 2004) and how to incorporate spatial heterogeneity in ecosystem functioning research (Turner & Chapin, 2005; Mokany et al., 2008).

To date, most of the experimental studies have been based on microcosms or mesocosms units, where diversity has been manipulated by randomly choosing some number of species from a pool of species, placing these together in experimental homogeneous units and then examining the relationship between species diversity and the ecosystem processes of interest. The spatial distribution of the species in experimental plots is typically random. However, in natural communities, spatial distribution of plants and animals is typically non-random and these spatial patterns

may be relevant to ecosystem functioning (Maestre et al. 2005). However, as Turner and Chapin(2005) underlined ecosystem functioning research still lacks of a spatial explicit theory and only recently some work have been done in the biodiversity-ecosystem functioning context (Bulling et al., 2008; Weis et al., 2008; Ericson et al., 2009).

Spatial models which include the spatial structure of communities show that species distribution influences many ecological processes (Bolker et al., 2003; De Boeck et al., 2006).This is particularly relevant for sessile organisms, where the patterns of intraspecific aggregation change the frequency and intensity of inter- vs. intraspecific interactions (Stoll & Prati, 2001; Tirado & Pugnaire, 2003). To date, there is little empirical evidence of the importance of spatial patterns to ecosystem functioning (Maestre et al., 2005). De Boeck et al., (2006) published a model that reveals functional consequences of the spatial arrangement of organisms. Recently, Orwin et al., (2014) performed an experimental work were the effects of spatial arrangement of plants on several ecosystem functioning proxies were evaluated.

In marine systems, Arenas et al., (2009) explored in natural assemblages the relationships between different diversity-related attributes and productivity in natural macroalgal assemblages. Specifically these authors examined the effects of spatial aggregation on primary productivity. However to our knowledge, no experimental studies have addressed this question explicitly.

In this chapter we used a novel approach to examine the functional consequences of an often overlooked aspect of biodiversity. We used synthetic assemblages and a fully factorial design with three different spatial patterns and two different morpho-functional richness levels (1 and 2 functional groups) to examine the effects of spatial aggregation in the primary productivity of macroalgal assemblages.

From terrestrial literature (De Boeck et al., 2006), (Mokany et al., 2008), (Orwin et al., 2014) and previous observational studies in seaweed assemblages (Arenas et al., 2009), our initial hypothesis is that more aggregated assemblages will have a lower performance than highly patchy assemblages. This is because interspecific species interactions are often less intense than intraspecific competition or even of opposite sign (intraspecific facilitation), resulting in larger interspecific/intraspecific interaction balances which should foster primary productivity in patchy assemblages.

5.2. Materials and Methods

5.2.1. Experimental procedures: Assemblages construction

In the present study we manipulated diversity, identity and patchiness of three morpho-functional groups: (i) sub-canopy (Sc); (ii) turf-forming (T) and (iii) encrusting coralline (C) algae. Each group was represented by a minimum of two species or even more in the case of the morpho-functional group turf. Species within functional groups were randomly selected among those most abundant in the pool of species at the study region. The choice was driven by the logistical constraint of having species that were enough abundant to create our experimental treatments. Species chosen were: i) *Chondrus crispus* Stackhouse and *Mastocarpus stellatus* (Stackhouse) Guiry as sub-canopy algae; ii) for the turf-forming morpho-functional group the turf-forming *Corallina elongata* J.Ellis & Solander with companion species, specifically *Jania rubens* (Linnaeus) J.V.Lamouroux, *Ceramium* spp., *Pterosiphonia complanata* (Clemente) Falkenberg and *Ulva* spp. and iii) for the encrusting we used mostly the red calcareous seaweed *Lithophyllum incrustans* Philippi, but also *Lithothamnium* sp. and the phaeophyceae *Ralfsia verrucosa* (Areschoug) Areschoug.
In our experiment we didn't separate the effect of the morpho-functional group from that of the identity of the species representing it, nonetheless it remains appropriate to test our hypothesis about the interactions between functional diversity and spatial aggregation of organisms.

To construct our synthetic assemblages of varying diversity and patchiness, we use a similar procedure to Arenas et al., (2006). In spring-early summer when the biomass of the seaweed assemblages is higher in this area (authors' personal observation), boulders covered by the species of interest were collected from low intertidal rock-pools at Areosa and praia Norte, Viana do Castelo (two rocky shores less than 2 km apart). Once at the laboratory, grazers were removed from the boulders using freshwater baths and stored in outdoor 100 L tanks with aerated filtered seawater, until their utilization (always less than a week). Using a commercial stone cutter boulders were cut into little cubes (3x3 cm^2 surface and 2 cm high), assuring that they were covered on top by either one of the species of interest or bare rock. The cubes were then assembled to create the experimental treatments.

Figure 5.1. Example of plate (Crust-Turf) once assembled

A total of 72 plates were constructed by fixing 16 cubes onto a PVC plate (19 x 16 x 1.5 cm) with quick setting cement and screws (Figure 5.1). To keep the cubes in place and to protect the whole assemblage, stripes of PVC (12x1x2 cm) were screwed on the plate fencing the group of the 16 cubes. Four replicates plates were allocated to each combination of the following factors: (i) Diversity (one or two morpho-functional groups present); (ii) Assemblage identity (the three single groups plus the three possible combinations of two of them) (iii) Patchiness (3 different combinations obtained by arranging the 16 cubes of the plate in half plate (Ha), quarters of plate (Qu), chess (Ch), as shown in Figure 5.2). Relative densities of each morpho-functional group present were maintained constant across all treatments (8 cubes) but overall density was double in the assemblages with two functional groups.

Once constructed plates were transported to Areosa beach and deployed at three contiguous large rock-pools (less than 5 m apart), at similar depth (between 20 and 30 cm). Attention was given to separate the plates (at least 1 m between two adjacent plates) and scatter them in the pools among treatments. The plates were attached to the substratum by screwing them in four holes done at the corners of the plate.(Figure 5.3)

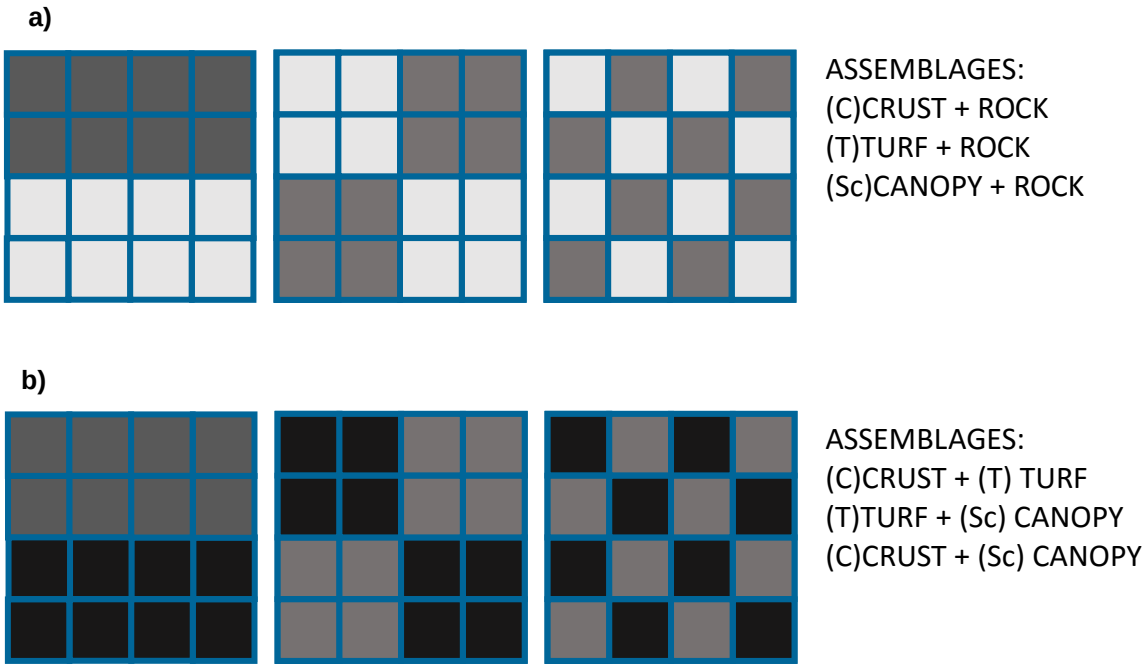

Figure 5.2. Spatial arrangements used in: a) single morpho-functional and b) bi-morphofunctional assemblages. Darker colours represents each different morpho-functional group. Light grey colour represents bare rock.

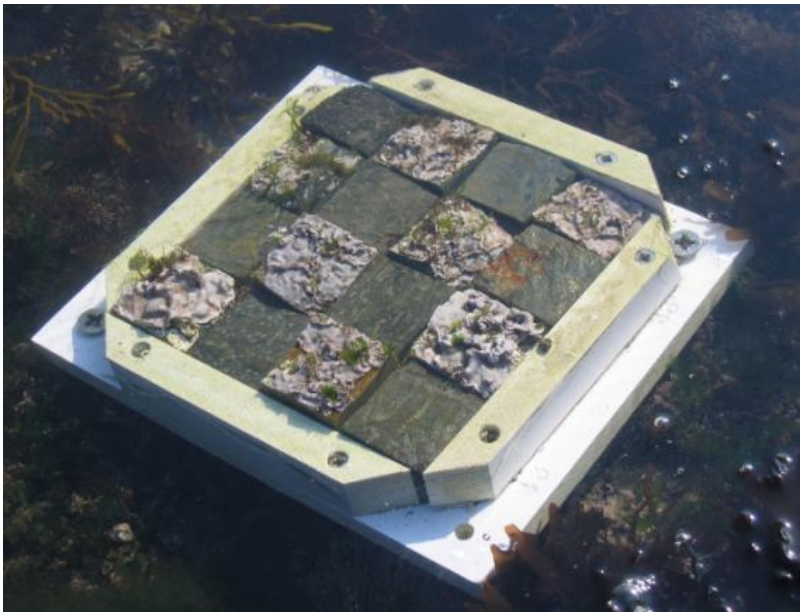

Figure 5.3.Crust -Bare rock plate attached to the rocky shore

5.2.2. Incubations procedures

In autumn, after four months in the shore and once the species in the assemblages were adjusted and accommodated each other, plates were transported back to the lab in plastic boxes cover by wet clothes. Once in the lab, grazers and other animals were carefully removed from the plates using freshwater baths and manual removal. Few ephemeral algal species that colonised some of the plates were also manually removed using forceps. Then plates were left for 24 h in an outdoor 300 L tank with aerated filtered seawater before the incubations to determine their functional performance. Functional proxies measured in this experiment were net primary productivity and respiration rates.

Total assemblage net photosynthesis (NNP) and respiration (RESP) were measured as the rate of oxygen increase or reduction in an incubation chamber placed in a CT chamber with controlled light and temperature (Arenas et al., 2009). GPP was calculated as the sum of the net photosynthetic rate and dark respiration (GPP=NPP+RESP), assuming that dark respiration remained constant during the light period (Binzer & Middelboe, 2005).

The incubation chamber consisted of a 15.2 l Plexiglas chamber partially submersed in a larger, thermostatically controlled 88 l cooling chamber, also Plexiglas. Seawater used for the incubations temperature inside the incubation chamber was kept always between 16 and 18 °C. Water movement inside the chamber was maintained through a submersible pump (1200 l h^{-1}) equipped with a diffuser to reduce turbulence. Incubations were performed inside a phytoclimatic chamber (Abalab® Fitoclima 750E) with light control facilities which allowed us to measure assemblage productivity at darkness and two successive light levels: 0 (dark), 260, and 1105 µmol m^{-2} s^{-1}, measured using a Biospherical® QSL-2000 Radiometer. The light source in the chamber were of eighteen 58 W fluorescent tubes (Osram® Cool White). (Figure 5.4)

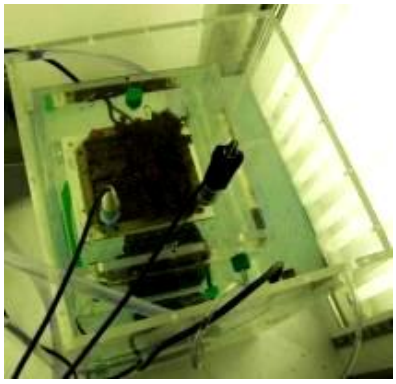

Figure 5.4. Incubation chamber used in this experiment

Dissolved oxygen concentration inside the chamber was measured using a HACH® luminescent dissolved oxygen probe. Probe signals were registered every minute and followed in a computer screen to ensure the linearity of the rates. Linear regressions were highly significant for all the incubations performed ($R2>0.9$, $p<0.01$ for all the incubations). Each incubation had a 30 minutes dark period followed by the two light periods with the same duration. pH was monitored in those incubations with higher biomass thought a HACH® pH probe to guarantee carbon availability in the water, pH values were always relatively constant. To minimise possible circadian effects all incubations were run at daytime between 9 in the morning and 4 in the afternoon. Incubations were run using Incubations were run using filtered seawater through a 5 µm filter and always within 48 hours after the arrival of the plates to the laboratory from the shore.

After the incubations, biomass (g dry weight) was estimated using non-destructive methods as in Olabarria et al., (2013). For the crustose, total cover of the plate was measured using digital photography and the dry weight was estimated from a surface-weight relationship previously calculated for each species using 20 patches of crust which were scraped and weighted after drying 24 hours at 60 ºC. For the other species (sub-canopy and turf) biomass was estimated from individual measurement maximum frond length (L) and basal and maximum circumference (C) and using linear regressions which estimated the relationship between length and the referred circumferences with the dry weight for each species. These regression were calculated using 40 fronds collected in the same area and dried for 48 h at 60 ºC.

5.3. Statistical analyses

A two way ANOVA was performed to test the hypothesis that patchiness has a positive effect on assemblages performance. Factors in the analysis were (i) Assemblage: fixed term with six levels (C, T, Sc, C-T, C-Sc and T-Sc) and (ii) Patchiness, fixed, 3 levels. Since assemblages was always a significant factor, we carried out a test effects of assemblages diversity, using partitioning of variances (Zardi et al, 2015). Thus the sum of squares (SS) from the linear model was partitioned into three orthogonal components to separate the effects of richness (mono-functional versus mixed assemblages) and identity within each assemblage diversity. Before each analysis, the assumption of homogeneity of variances was assessed using Cochran's *C* test

(Underwood,1997). In case of non-homogeneity, appropriate data transformations were applied. SNK (Student – Newman – Keuls) tests were used to make an *a posteriori* comparison among means.

5.4. Results

Table 5.1 shows the analyses of variance for the three primary productivity proxies measured in the experiment. Assemblage's morpho-functional type and functional richness were significant factors resulting from differences in species-specific photosynthesis rates and on total standing biomass.

Table5.1.Analysis of variance for the functional proxies measured in the experiment: a) Gross primary productivity, b) Net primary productivity and c) Respiration rates. Bold indicate significant terms at p<0.05. To simplify, we did not include in the table "within diversity level" factors. See more details on M&M regarding the use of partitioning of variance techniques to examine diversity effects.

		a) GPP		b)NPP		c)Respiration	
Sources	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Assemblage	5	35.2	**0.000*** **	35	**0.000*** **	8.88	**0.000*** **
Funct.richness	1	43.9	**0.000*** **	43.9	**0.000*** **	9.98	**0.003** **
Patchiness	2	3.99	**0.024***	4.22	**0.020** **	2.74	0.073
AssemXPatch	10	0.93	0.517	0.96	0.488	0.88	0.558
Error	54						

Differences in performance among morpho-functional types are easily noticeable in figure 5.5. Encrusting species had the lowest performance values, while sub-canopy species were the most productive. These functional differences were mirrored in the assemblages with two morpho-functional groups.

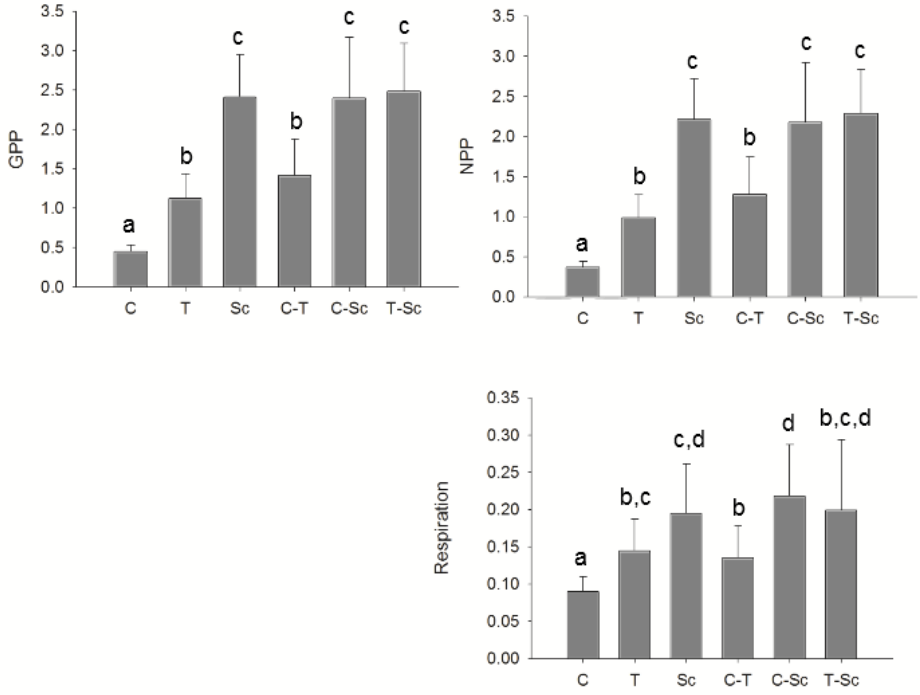

Figure5.5.GPP, NPP and Respiration rates for all the single morpho-functional groups included in the experiment. (C) Crust indicates encrusting species, (T) Turf: turf-forming species, (Sc) Subcanopy: sub-canopy species, C-T: Crust-Turf, C-Sc: Crust-Subcanopy and T-Sc: Turf- Subcanopy.

Probably, the most interesting finding was the fact that patchiness was significant for gross and net primary productivity. Higher patchiness increased productivity in our assemblages and this pattern was consistent for all the assemblages and functional richness. This increase was consistent across assemblage identities. Primary productivity of the highly patchy assemblages (i.e. chess arrangement) was more than 15% higher than those assemblages with a large single patch (Figure 5.6). No effects significant effects were found in the case of respiration suggesting that the effect of patchiness is light dependent and probably linked with a better efficiency of the assemblages less clumped.

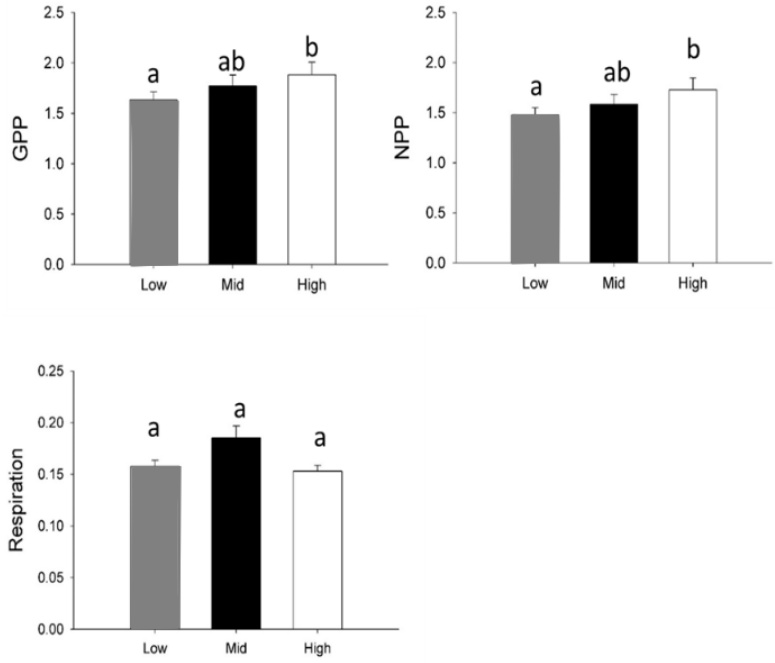

Figure 5.6. Effects of spatial arrangement patterns on GPP, NPP and Respiration (all in µmol O_2 min^{-1}assemblage^{-1}). Low - low patchiness, Mid – Mid patchiness and High – High patchiness. Letters above the bars indicate the groping results from SNK tests. Bars sharing the same letter were not significantly different at p=0.05.

5.5. Discussion

Spatial heterogeneity is relevant to ecosystem functioning at multiple scales, from centimetres to the globe (Lovett, 2005) and patch structure is one of the components of spatial heterogeneity (Strayer, 2005). Here we examined small scale patchiness effects on assemblage's productivity. Like in terrestrial systems, species in marine ecosystems are not homogeneously distributed, neither in biomass or spatially. Our results confirm that local scale heterogeneity of communities, i.e. the spatial aggregations of individuals may have very relevant functional implications.

The spatial distribution pattern of organisms is potentially an important attribute that has been overlooked by biodiversity–function research (Maestre et al., 2012). In terrestrial communities, existing evidences show that spatial aggregation of species may affect the productivity of plant communities (Maestre et al., 2005; Mokany et al., 2008). For instance, (Maestre et al., 2012) found that ecosystem performance was maximized under a random spatial pattern in soil crust communities dominated by lichens.

On the marine realm, studies on spatial heterogeneity and patchiness are not new. There is a large body of literature on the spatial structure and variability of intertidal assemblages, see for instance (Underwood & Chapman, 1996; Benedetti- Cecchi, 2001, Fraschetti et al., 2005; Coleman et al., 2006; Burrows et al., 2009). From this large bunch of research it is obvious that small scale variability is a general attribute of benthic marine coastal assemblages and patchiness is a general feature from centimetres to meters (Fraschetti et al., 2005). In fact, marine benthic ecologist have been largely interested on the complex set of physical and biological processes that shape this spatial structure (Benedetti-Cecchi & Cinelli,1992; Benedetti-Cecchi & Cinelli,1996; Denny et al., 2004; Benedetti-Cecchi et al., 2006). However the functional consequences of assemblages' spatial structure have been largely ignored. Under the biodiversity-ecosystem research topic, some studies on benthic marine systems have shown that physical heterogeneity can modulate diversity effects (Griffin et al., 2009) but see (Weis et al., 2008).Most of these studies have manipulated the degree of environmental heterogeneity and examined how it affects the relations between biodiversity and functioning. Our approach was largely different. We manipulated not the heterogeneity causes but the heterogeneity effects, i.e. heterogeneous distribution of species. We carried out our study in relatively homogenous conditions, using

artificial assemblages mimicking rockpool communities where intertidal emersion stress is partially hampered. Our design with a very limited diversity gradient, only two morpho-functional groups in the richest treatment was not the best to test for diversity effects. The design used intended to examine specifically the effects of species patchiness avoiding any confounding with other diversity-related processes. In fact in biodiversity experiments spatial aggregation could be having a hidden effect since, in the substitutive designs usually used, low diversity assemblages would naturally have a higher spatial aggregation than high diversity treatments (Arenas et al., 2009).

We included three very different morpho-functional groups present in these habitats, ranging from encrusting species, turf-forming and small canopy species. We include encrusting seaweeds, often a neglected group on these type of experiments using synthetic assemblages (but see (Arenas et al., 2006; Vaz-Pinto et al., 2014) because their relevance in terms of cover in rockpools. In fact the three groups cover most rockpools substrate in the area of study (authors per. observations). By including these very different groups we increased the probability of increasing trait-dependent complementarity among the species and therefore the influence on both functional groups diversity and patchiness (Bruno et al., 2006). We created relatively simple, regularly spatial aggregation patterns which can give rise to general hypotheses about heterogeneity that can be extended to or tested in other systems (Strayer, 2005).

The effect of species aggregation of macroalgal assemblages on primary productivity was examined in an observational study on natural assemblages by Arenas et al., (2009). These authors found a negative statistical relationship between the clustering of the most abundant species on the primary productivity of the whole assemblage, suggesting that clustered dominating species would reduce the overall performance of the assemblages. However observational studies do not allow to establish causal relationships.

To our knowledge, this study is the first one to examine experimentally the effects of patchiness on the productivity on macroalgal assemblages. Given the evidences from previous experiments in terrestrial systems and the findings found on observational studies, the increase of productivity with higher patchiness was not a novel finding, but the magnitude of the increase was quite a surprise. Highly patchy assemblages were around 15 % more productive than assemblages with only one large patches, and this

patterns was consistent across the different morpho-functional identities included in the experiment. This effect has a magnitude similar to those described for species richness and suggests that aggregation patterns resulting from usually spatial heterogeneity should not be neglected in biodiversity-ecosystem functioning studies.

In seaweeds like any other sessile organisms, patterns of intraspecific aggregation change the frequency and intensity of inter- vs. intraspecific interactions (Stoll & Prati, 2001), modifying the efficiency in resource use and ultimately enhancing the productivity. Our findings provide the first explicit experimental evidence in macroalgal assemblages that spatial arrangement may mediate other diversity-related effects. We think this is highly relevant in a system like macroalgal communities which have a strong prevalence of spatially heterogeneous processes generating small to large scale heterogeneity patterns.

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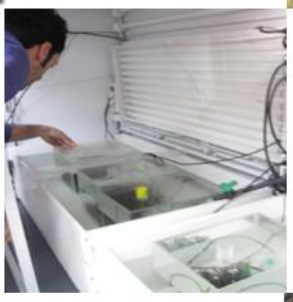


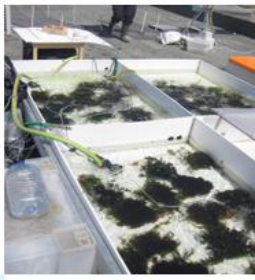

Part VI

Chapter 6

General discussion

"The first law of ecology is that everything
is related to everything else."
Barry Commoner, 1971

6.1. General discussion

Research into the links between biodiversity and ecosystem functioning began in the 1980s and since them the functional consequences of the global loss of diversity have received considerable attention by researchers. As a result, the topic of biodiversity and ecosystem functioning has now emerged as a major field within ecological research and is considered new ecological paradigm (Gamfeldt and Hillebrand 2008; Naeem, 2002). In the last two decades, major international research initiatives and projects were carried out in this new area of knowledge. Hundreds of experiments were performed in ecosystems all over the globe, resulting in novel ecological ideas which were tested experimentally.

These results has provided increasingly rigorous answers to the question of how and why the Earth's biological diversity influences the functioning of ecosystems. Nowadays, from extensive body of research some strong evidences emerged. In a recent review of this research, spanning different ecosystems and conditions, Cardinale (2012) concluded that: "*There is now unequivocal evidence that biodiversity loss reduces the efficiency by which ecological communities capture biologically essential resources, produce biomass, decompose and recycle biologically essential nutrients".*

Ecosystems also provide a multitude of benefits to mankind, from food, clean water or protection, to name a few of the known as Ecosystem services. Ecosystem services are the many different benefits that ecosystems provide to people (Millennium Ecosystem Assessment, MA, 2005). However, many of these benefits are under severe threat from man-made pressures. For example, it is estimated that 60% of the world's ecosystems are degraded or used unsustainably; 75% of fish stocks are over-exploited or significantly depleted and 13 million hectares of tropical forests are cleared each year (FAO, 2011). Hence, understanding the relevance of biodiversity on ecosystem processes is essential to disentangle direct effects of species loss from of other global impacts like climate change which are also driving diversity changes and altering ecosystem function.

Marine research on biodiversity-ecosystem functioning lagged behind the terrestrial counterpart, however in a recent meta-analysis of the research done, Gamfeldt et al.,2015) suggested that changes in the number of species tend to alter the functioning of marine ecosystems in a similar way to terrestrial systems. These authors identified

some of the current gaps in the field of marine biodiversity–ecosystem function research. For example they highlighted the need to further explore the direct, indirect and interactive effects of changes in diversity. Also we still have a relative low understanding on how natural mechanisms driving diversity patterns in marine systems affect the effects of diversity on the functioning of natural systems. In other words, it is precise to move from laboratory experiments and get closer to those mechanisms occurring on natural communities.

We used marine seaweeds assemblages to begin this "laboratory to natural system movement". Seaweed assemblages (at least in intertidal zones) are easy to manipulate, thus they are a convenient model system for experiments examining causal links between diversity and functioning. Several authors have performed studies involving seaweeds within the context of biodiversity ecosystem functioning research measuring processes such as primary production, nutrient uptake, resistance to invasion and stability (Mineur et al., 2015). These experiments mostly found positive effects of seaweed diversity, irrespective of the response measured: photosynthetic rates (Bruno et al., 2005; Griffin et al., 2009; Middelboe and Binzer., 2004), biomass accumulation (Bruno et al.,2005), nutrient uptake (Bracken and Nielsen, 2004; Bracken and Stachowicz, 2006), invasion resistance (Arenas et al., 2006) and stability (Boyer et al., 2009; Stachowicz et al., 2008).

Seaweeds assemblages are not just a good model system. They are primary producers of capital importance in the marine coastal areas dominating rocky shallow coastal areas where most of the bottom lies within the euphotic zone and supporting highly productive benthic communities (Mann, 1973). Estimates of NPP on a global scale based on a number of studies suggest that terrestrial ecosystems can produce between 100-1500 g C m-2 yr-1 (Hazarika, 2005; Chen, 2012; Yoshio, 2004). Meanwhile, (Tait, 2010) suggest an annual average productivity of the macroalgal assemblages up to 3 times more, between 700-5000 g C m^{-2} yr^{-1}. Large canopy species like *Ascophyllum nodosum*, *Macrocystis integrifolia*, *Sargassum horneri*, *Postelsia capillaceae* and *Ecklonia radiata* have productivity rates above 1000 g C m^{-2} $year^{-1}$ (Chung, 2010). Seaweed beds, particularly provide food and shelter for associated fauna like sea-urchins, amphypods, but are very important for the recruitment and protection of many commercially important fish and shellfish fisheries

(Graham, 2004). Besides the direct consumption by grazers, other organisms like filter feeders benefit from seaweed-derived particulate matter. When seaweeds decay, they give off a large amount of organic matter that is physically or biologically degraded and becomes a very nutritious food for marine fauna (Duggins et al., 1989). Isotopic analyses showed that indeed 60–85% of the food of filter-feeders came from particulate subtidal seaweeds (Bustamante, 1996). Some of these services are at risk. In our shores, seaweeds communities are under large pressures derived from the arrival of new introduced species (that already account for 5–10% of the European seaweeds) and the regional extirpation of native species resulting from oceans' climate change and anthropogenic local stressors like pollution or harvesting. These structural changes are creating new diversity scenarios with undetermined functional consequences (Mineur et al., 2014).

The experimental approaches presented here brought in our opinion new light on how components biodiversity and diversity promoting mechanisms influence the functioning of seaweed assemblages. For instance, it is well acknowledge than species richness may enhance ecosystem processes, but evenness effects are less studied. It has been predicted that the relative abundance of species may change the intensity and even the direction of the relationship between diversity and community functioning (Nijs, 2000). In fact, despite the usual identification between diversity and species number (Lepš 2013), the number of species itself may not a satisfactory descriptor of community diversity and a very poor predictor of ecosystem functioning. In the experience described in chapter 2, we found significant evenness effects in the seaweed assemblage’s communities that were mediated by richness. Thus, decreasing evenness reduced productivity deeply in depauperate communities with a reduce number of species. This is a remarkable finding since highly perturbed systems have frequently few species and low evenness and from our experiments result they would also suffer an unpredicted reduction in productivity.

Nevertheless, and above any other structural driver, primary productivity was much shaped by the species traits included in the different assemblages. There is nothing novel in this finding, species traits is recognized as the most relevant diversity component determining ecosystem processes (Lepš, 2004; Gamfeldt, 2008; Harvey, 2013; Bruno, 2005). Some authors explain that in short-term experiments the identity appeared to be the dominant effect (Bruno et al., 2006; Griffin et al., 2009), while in

longer experiments mechanisms like facilitation and differential use of resources increase in importance (Stachowicz et al., 2008).

It is unlikely that specie traits effects will be overcome by non-additive effects resulting from species interactions. There have been for some years some debate on whether species traits or species diversity are more relevant to ecosystem functioning. This debate is largely a false dichotomy. Increasingly, the evidence shows that both the number of species, their interactions and types of species in an ecosystem impact biomass production (Enquist et al., 2015).

It is not just species traits, number of species or evenness. In the case of sessile organisms, spatial aggregation of species should also be considered as a driver of functioning as any of the other structural traits. Chapter 5 uses an innovative approach to show how patchiness may promote productivity on macroalgal assemblages. This is the first study in the marine system that explores patchiness in the context of biodiversity-ecosystem functioning research. The magnitude of the effect was quite high and emphasize the relevance of the effects of species aggregation on the ecological processes. Like in the case of evenness, impacted assemblages have usually a low richness and often highly aggregated species which could intensified the effects associated to low richness.

In our aim to bring real processes into biodiversity-ecosystem functioning research, we examined how two different natural diversity-promoting mechanisms could affect the relationships among structural traits like species richness or evenness with seaweed community performance. The first mechanism scrutinized was disturbance. While the effect of disturbance on seaweed assemblages has been very well examined, there are few similar experimental approaches which have tried to measure in situ functional response, i.e, primary productivity. Our two experiments showed different results but pointing a similar idea: at our scales of study there is an uncoupling in the relationship between structure and productivity in these type assemblages. Hence in the first experiment, the structural changes created by the disturbance did not result on functional changes on the assemblages and in the second result were the opposite with no structural changes but gross primary productivity was higher in perturbed plots than in controls.

Thus, natural rockpool assemblages like those used in our experiments were very resistant both structural and functionally to disturbance. This lack of relationship may result from the narrow range of species richness found in our experiment, but also a consequence of the relative low importance of species richness and species interactions in highly dynamic assemblages like rockpools where species coexist through colonization-competition trade-offs (Shurin & Allen 2001); (Kinzig & Pacala, 2001).

The second mechanisms examined was successional diversity. Despite recent efforts to use a functional framework to explain ecological succession (Raevel et al., 2012) we are not aware of any study looking into the functional consequences of succession within the Biodiversity-Ecosystem Functioning research context. In the experiment described in chapter four we found that the different successional mechanisms prevailing along the successional process shape the relationships between diversity and species richness or evenness. Colonization-competition trade-offs driving primary succession seems to uncouple richness-productivity relationship, a similar result to those reported in the perturbation experiments. Only species traits seemed relevant in this successional context with large effects of early colonizers with high productivity rates like *Ulva* spp. In the case of secondary succession, interactions among species, which increased in intensity along time finally created the expected positive relationship. Both, primary and secondary succession results agreed with the predictions made by the models of Kinzig & Pacala (2001) on the different roles of species interactions in shaping diversity-functioning relationships in primary and secondary succession. Finally, integrating all the experimental data in one set we can noticeably observe a positive diversity trend, probable similar to the trends that we could measure at landscape level studies (see Arenas et al., 2009), where multiple succession stages and environmental conditions simultaneously shape the relationship between diversity and functioning through the process of succession.

The functional approach used throughout this report and the results obtained in our experiments open some lines of future research. It is obvious that biodiversity has a fundamental role to play in ecosystem functioning, underpinning essential processes such as resource capture, biomass production among others. The work developed help to understand how and when biodiversity is relevant in shaping primary productivity of seaweed assemblages. It is now time to further understand how global scale threats

like climate change or invasions may affect biodiversity–ecosystem functioning relationships (Gamfeldt et al., 2005).

To conclude, the importance of biodiversity can be summarized in two essential features. On the one hand biodiversity is the fruit of labor of millions of years of nature, so its value is immeasurable and irreplaceable. On the other hand, the diversity of species is a guarantee for the proper functioning of natural systems. Hence, we can say that biodiversity concerns are not only about keeping species, but because biodiversity is essential for the functioning of the ecosystem and therefore the preservation, conservation, and restoration of biodiversity should be a high global priority.

6.2. References

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